GENETIC CONTRIBUTIONS TO THE CLASSIFICATION OF RENAL CELL CANCER

RIJKSUNIVERSITEIT GRONINGEN

GENETIC CONTRIBUTIONS TO THE CLASSIFICATION OF RENAL CELL CANCER

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aan mijn ouders en René

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Cover: "Progression"

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VOORWOORD

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CHAPTER 1

1 GENERAL INTRODUCTION

CHAPTER 1

GENERAL INTRODUCTION

Cancer, chromosomes and genes

In limited time, a single fertilized egg gives rise to a complex multicellular organism consisting of differentiated cells arranged in a precise pattern. During embryonic development the different cell types become determined, each in its proper localization. The adult human body is a stable ecosystem in which one generation of cells succeeds another. A continuous process of cell proliferation is necessary throughout life to replace cells that have been lost due to injury or death, or to maintain populations of different cells that have short lifespans. In a normal situation there is a precise balance between cell proliferation and cell death.

A normal cell has multiple independent mechanisms to control growth and differentiation, encoded in genes in its DNA. Each DNA molecule is packaged in a separate chromosome, and the total genetic information stored in the chromosomes of an organism is said to constitute its genome. A typical human cell contains 46 chromosomes, consisting of 23 pairs, one member of which is inherited from each parent. Each gene is therefore present in twofold, except for X- linked genes in males.

Cancer is a caricature of developmental biology, caused by mutations in genes that control cell growth and differentiation. There are three classes of genes which, when altered by mutations, will contribute to the development of cancer. These are proto-oncogenes, tumor suppressor genes, and mutator genes. Normal or "wild-type" genes in all three categories affect the process of cell division or the fidelity of DNA replication. The products of proto-oncogenes act to stimulate normal cell proliferation. Oncogenes may arise from proto-oncogenes through a variety of events, including point mutations, multiplication (dose effect), and juxtaposition to other chromosome sequences. Oncogenes act in a dominant fashion. Tumor suppressor genes have a growth inhibiting function in the normal situation, and contribute to malignant growth through loss rather than activation. Their behavior is recessive, and both copies must be inactivated for tumor formation to occur. This inactivation may result from small deletions or point mutations, but also from loss of whole chromosomes. The so called mutator genes are genes that in normal cells control the repair of DNA damage, maintain the fidelity of DNA synthesis and regulate proper cell division. Loss of function of these genes leads to a destabilization of the genome, thus facilitating the formation of additional genetic changes. Analogous to tumor suppressor genes, mutator genes behave in a recessive fashion, meaning that both gene copies have to be inactivated before destabilization occurs.

The development of cancer is a multistep process in which a single initially altered cell becomes malignant through a series of gradually progressive changes. Most cancers are clonal in origin, but tumor progression, i.e. acquisition of the capacity to invade and to metastasize, involves sequentially acquired genetic changes within the evolving neoplastic clone, leading to subpopulations with more aggressive growth characteristics. Therefore, despite their clonal origin, malignant tumors tend to be heterogeneous, comprising several genetic subpopulations of cells with different biological properties, regarding invasive and metastatic potential, and sensitivity to

therapy. Chromosome aberrations in cancer cells can be divided into primary and secondary aberrations. Primary chromosome aberrations are directly related to tumorigenesis. They may occur as the sole abnormality and are often specifically associated with distinct tumor types. Secondary chromosome changes do not appear as the sole abnormality and act towards progression rather than tumor initiation. However, secondary changes do not occur randomly and their appearance may depend on the primary aberration and on the specific tumor type involved.
Cytogenetic analysis of human cancer cells has yielded a huge amount of information about the incidence and nature of chromosomal abnormalities in malignant cells [1] Specific chromosome changes have been identified in several types of cancer, and provide important tools in the diagnosis of these neoplasms. Notable examples in solid tumors are t(X;18) in synovial sarcomas, t(11;22) in Ewing' sarcomas, and t(12;16) in myxoid liposarcomas. Molecular characterization of the loci involved in the specific translocations, has led to the isolation of several oncogenes, tumor suppressor genes, and tissue specific genes. Secondary changes, associated with tumor progression, can reveal information relevant for the prognosis.
Taken together, cytogenetic and molecular genetic analysis of tumor cells have, and will continue to provide essential information about the oncogenesis, progression, diagnosis, and prognosis of cancer.

Kidney development and function

The mature human kidney is a bean-shaped organ, which is on average 12 cm in length, 6 cm in width, and 2.5 cm in thickness [2]. It serves to convert over 1700 liters of blood per day into about 1 liter of a highly specialized concentrated fluid called urine [3]. In so doing, the kidney excretes the waste products of metabolism, precisely regulates the body's concentration of water and salt, maintains the appropriated acid balance of plasma, and serves as an endocrine organ, secreting such hormones as erythropoietin, renin, and prostaglandins. The physiologic mechanisms that the kidney has evolved to carry out these functions require a high degree of structural complexity.
Since the anatomy of the kidney has been detailed in several publications, only those features relevant to the understanding of renal cell cancer are emphasized [2]. Since renal cell cancer is commonly accepted to arise from cells of the mature renal tubular system, the structure of these functional units is explained. A mature uniferous tubule consists of a nephron (including a glomerulus and Bowman's capsule, a proximal convoluted tubule, loop of Henle, and distal convoluted tubule), and a collecting tubule. These two structures develop as separate entities from two different sources; the nephron develops from the metanephric mesoderm (metanephros), whereas the ureteric bud (mesonephros) is the primordium of the collecting tubule [4]. Mutual induction makes the ureteric bud grow and bifurcate and so form the collecting duct system, while the metanephric mesoderm is induced to move down the nephrogenic pathway [4]. When the distal part of a nephron contacts an arched collecting tubule, the two tubules become confluent and form a mature uniferous tubule (Figure 1). About 12 generations of nephrons form between the 8th and 34th week of gestation. By then kidney formation is completed and all renal growth from this point onward is the result of the enlargement of existing structures [2].

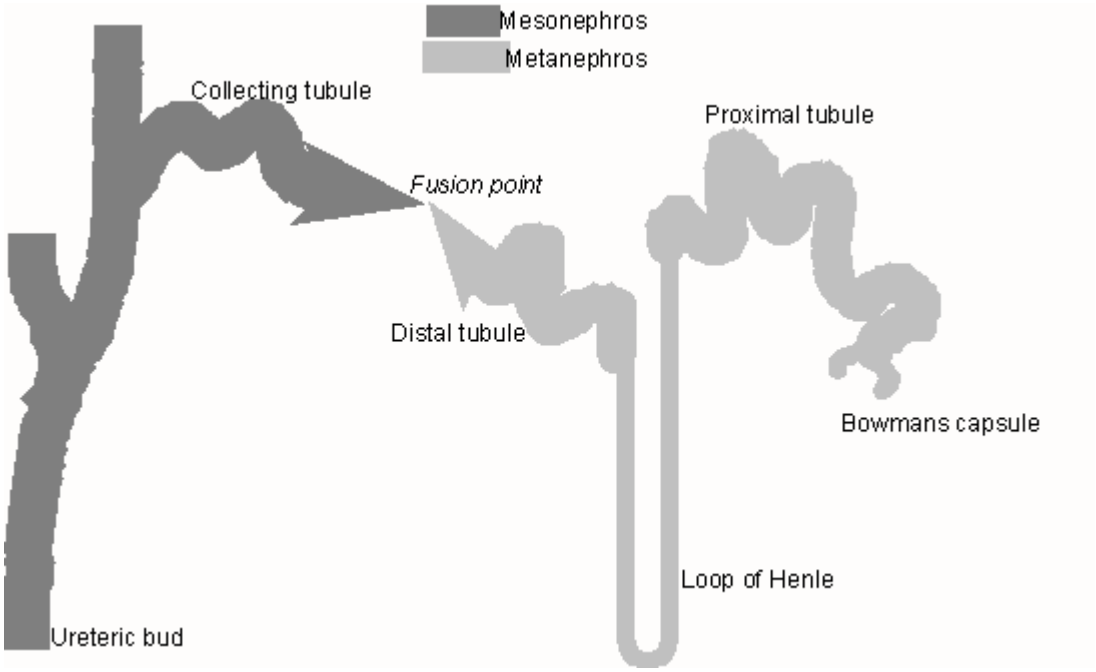

Figure 1: Schematic representation of the development of a mature uniferous tubule.

Renal cell cancer

Renal cell cancer comprehends a heterogeneous group of tumors which account for 2% of all cancers diagnosed. They comprise 80-85% of all malignant kidney tumors and affect males twice as much as females. The overall incidence increases with each decade of life showing a peak in the sixth decade. In rare instances, RCC affects children and young adults [5-9]. No clear-cut geographical or ethnic preference has been reported for RCC, although the incidence is higher in Scandinavia and the United States as compared to Asia and Africa [10].

RCC is identified in patients with end-stage renal disease at a rate six times greater and at an age more than 10 years younger than in the general population [11]. A higher incidence of RCC has also been reported for patients with acquired cystic kidney disease, and tuberous sclerosis [12]. Environmental factors contributing to the development of RCC are smoking, particularly in men, and obesity, especially in women [13,14]. Occupational exposure to various hydrocarbons (gasoline, petroleum, and tar and pitch products), and asbestos also increases the risk on having this disease [15].

A lack of early warning signs is characteristic for RCC. Small, localized tumors rarely produce symptoms and therefore the diagnosis is often delayed until after the disease is advanced [3,16]. Classic diagnostic symptoms associated with RCC are hematuria (in 50 to 60 % of patients), abdominal pain (in 40 %), and a palpable mass in the flank or abdomen (in 30 to 40 %). A combination of these three is found in only 10% of patients, and usually indicates advanced disease.

About 25-30% of patients present with metastatic disease at initial diagnosis, with lungs, bone, liver, and brain being the favorite sites.

RCC is a highly unpredictable neoplasm with a tendency to recur or progress and cause death many years after initial treatment [17]. Pathologic stage is probably the single most important predictor of prognosis [5]. Although a TNM staging exists for RCC [18], the Robson system remains the most widely used [19]. Five year survival ranges from 65-85% for stage I patients, 45-80% for stage II, 15-35% for stage III, and 0-10% for stage IV patients. Among microscopic features, assessment of nuclear grade has the greatest prognostic significance [5,20]. In addition, Störkel and associates [21,22] developed a prognostic score based on the prognostic potential of the following parameters: TNM staging or Robsons staging, grading, cell type, growth pattern, and patient age. Although a correct prognosis of over 80% was achieved on the average for an individual case, this system has not been widely adapted.

Surgical resection is the treatment of choice for RCC. In case of localized disease, radical nephrectomy is usually curative. Partial nephrectomy has been attempted in patients with bilateral tumors and in patients with only one functional kidney. The overall survival of these patients has been found to be similar to that of patients with disease of similar stage who undergo radical nephrectomy. Therefore, nephron sparing surgery is presently also performed on patients with small lesions (<4cm) and a normal contralateral kidney [16]. Once the disease has spread, no adequate treatment is available, since hormonal and chemotherapeutic agents have little or no effect [16].

The classification debate

The classification of renal cell cancer has traditionally been based on the cytologic and architectural patterns of growth. The World Health Organization (WHO) nomenclature for kidney tumors includes benign and malignant tumors and is restricted to adenomas and carcinomas, the latter being divided into papillary and nonpapillary growth patterns. All other tumors are grouped under "others". In describing the architecture, the histological pattern (acinar, tubular, cystic, sarcomatoid, papillary, solid) and cytological features (clear, granular, oncocytic, pleomorphic/spindleshaped) are often included [10]. This classification does not allow extensive subtyping and gives little insight in the oncogenesis, tumor progression, and clinical behavior of RCC [23].
In 1986 Thoenes and Störkel [20] introduced a new, refined, classification for RCC, based on morphological, histochemical, and electron-microscopic data. A total of five basic subtypes of RCC are recognized, related to their origin from different parts and cells of the nephron (Figure 2). An extensive description of the morphological and genetic features of the different subtypes will be discussed in chapter 5. The cells of the proximal part of the renal tubule give rise to clear cell and chromophilic RCC, comprising 70-75% and 10-15% respectively. Chromophobe RCC (2-5%) and renal oncocytoma (4%) are derived from the intercalated cells of the collecting tubule, whereas Duct Bellini carcinomas (1%) find their origin in the principal cells of the medullary collecting duct. Variants can be assigned to most of these subtypes, resulting from an accumulation of mitochondria (eosinophilic variants). Sarcomatoid transformation may occur in any of the subtypes, except for the benign oncocytomas and represents an ultimate form of dedifferentiation. Sarcomatoid RCC usually is composed of sarcomatous and carcinomatous areas, and the diagnosis is made on the

properties of the carcinomatous component. When the entire neoplasm has a sarcomatous appearance, a correct diagnosis is difficult to make.

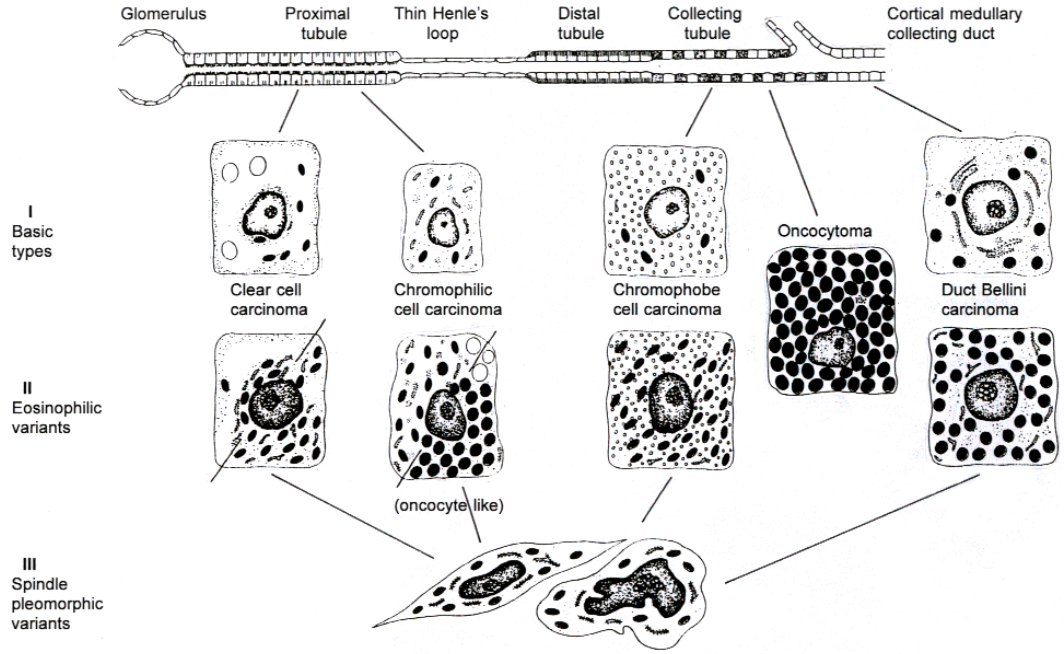

Figure 2: Cytomorphological classification of renal-cell carcinomas (according to Thoenes and Störkel) in relation to the nephron and its cell types [20].

Three growth patterns are distinguished: solid, tubulo-papillary and cystic. Generally, one growth pattern predominates in a given tumor. A relation exists between cell type and growth pattern, but this is not an exclusive one. Clear cell and chromophobe RCC mainly have a solid growth pattern. Chromophilic RCC predominantly shows a tubulo papillary architecture and renal oncocytoma is related to acinar growth. The Duct Bellini carcinomas are associated with both a compact and a tubulo-papillary growth pattern. Renal tumors are graded according to nuclear morphology, including size of nucleoli supplemented by cytoplasmic features, recognizing G1, G2, and G3/4 tumors.
In 1995 this classification has been updated with the introduction of two new subtypes of RCC: neuro endocrine RCC and metanephroid renal adenomas [24]. These tumor types are rare entities and comprise less that 1% of RCC each. Since histological data concerning these subtypes is extremely scarce and no genetic data are available, these subtypes are excluded from the present study.
Since the introduction of the classification of Thoenes and Störkel, their morphological subtyping has been validated by several cytogenetic and molecular genetic studies, showing distinct combinations of genetic changes present in each of the subtypes mentioned above. Hereditary and sporadic

cases of clear cell RCC are characterized by deletions of the short arm of chromosome 3. Chromophilic/papillary tumors show a unique combination of autosomal gains: i.e. +7,+12,+16,+17, and/or +20. Chromophobe RCC is characterized by extensive non random chromosome losses, involving chromosomes 1, 2, 6, 10, 13, 17, 21, and the X or Y chromosome. Renal oncocytomas show a variety of chromosomal patterns, from which two genetically distinct subsets seem to emerge. One subset consistently shows the combined loss of chromosomes 1 and X/Y, whereas the other reveals a translocation involving breakpoint 11q13. In addition, both renal oncocytomas and chromophobe tumors exhibit changes in their mitochondrial DNA (mtDNA) and show telomere shortening and telomeric associations (tas). Up till now Duct Bellini carcinomas have been scarcely studied. Preliminary data indicate that loss of 8p and 13q may be important in their development.

Aim of the thesis

The histopathological classification of renal cell cancer is a constant matter of debate. This is not surprising, since renal cell cancer comprises a heterogeneous group of tumors, the phenotype of which may change dramatically during progression. On the other hand overwhelming evidence is available on the existence of genetically distinct subtypes of RCC. A specific combination of genetic changes marks each of the subtypes, whereas other changes have been related to progression. The generation of a genetic classification of RCC has been advocated by Kovacs and by us for several years [23,25-28]. The great advantage of such a genetic classification would be that genetic changes, heritable through cell division, are constant during tumor progression.
Since genetic changes influence the morphology and biological behavior of tumor cells, there is a direct relation between both. However, the widely used WHO classification allows only subdivision into adenomas, carcinomas, and others. This classification does not do justice to the great diversity in genetic constitution, phenotypic appearance and biological behavior of renal neoplasms. The classification of Thoenes and Störkel, introduced in 1986, in which he different RCC subtypes are related to different cells and parts of the nephron, allows a more extended and refined subtyping, which may coincide with genetic subtyping. In addition the genetic constitution may lead to a refinement of the classification.
In order to elucidate whether, and to what extent the morphological classification of Thoenes and Störkel correlates with the observed genetic subsets of RCC, we combined the genetic data and morphological features of a large number of renal cell tumors, including own data and data extracted from the literature. In addition, we aimed to clarify possible pathogenetic relationships of different RCC subtypes and tried to elucidate the different oncogenetic steps important in the development and progression of these neoplasms, focussing mainly on the subtypes other than clear cell RCC. This survey is an extension of the data presented in the thesis of Dr. E. van den Berg-de Ruiter in 1993. The results of the present survey, as well as results from the literature, are compiled in a new oncogenetic model for renal cell cancer, depicted in chapter 5. The question is addressed whether the different genomic abnormalities found in the different subtypes of RCC may serve as tools for classification, and the identification of histogenetic relationships, and whether they will support, refine and/or change classification systems. The genomic alterations mark the location of

genes involved in the oncogenesis of renal cell tumors and may finally lead to their isolation and identification.

CHAPTER 2

2. GENETIC ANALYSIS OF RENAL CELL CANCER, AN OVERVIEW

CHAPTER 2

CYTOGENETIC CLASSIFICATION OF RENAL CELL CANCER

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Cancer Genetics and Cytogenetics: in press

INTRODUCTION

Cytogenetic and molecular genetic investigations in cancer are important tools to address problems of oncogenesis and tumor progression, of classification and of diagnosis of tumors. Combination of advanced molecular genetic, cytogenetic, and (immuno)histopathological analysis will contribute significantly to the elucidation of the oncogenic steps that lead to immortalization and subsequent malignant behavior.
In this review written on the occasion of Dr. Avery Sandberg's 75th anniversary, we will present a model for the pathogenesis of renal cell tumors based on a new cyto-morphological classification and our (cyto)genetic analysis of about 175 renal cell tumors, together with the accumulated data in the literature.

RENAL CELL CANCER

Renal cell adenomas (RCA) and carcinomas (RCC) constitute a heterogeneous group of tumors and this heterogeneity is a well-known complicating factor in the diagnosis. The histogenesis of renal cell cancer has been controversial for a long time, especially in terms of the thesis originally expressed by Virchow and advocated by Grawitz [29] that certain clear cell epithelial renal tumors are derived from ectopic adrenocortical elements. This has led to the term "hypernephroma" or Grawitz tumor and finally also to the term "hypernephroid renal carcinoma". Although true hypernephrogenic tumors do rarely occur in the kidney, the hypernephrogenic theory of the clear cell epithelial renal tumors has been questioned for a long time and preference is given instead to the renal tubular histogenesis. The evidence that the usual (nonembryonic) renal adenomas and carcinomas in all their variants derive, in principle, from the mature uriniferous tubule has been promoted and consolidated by animal experiments with carcinogens and observation of pre-stages and early stages of epithelial renal tumors in human kidneys [3,19,30].

CLASSIFICATION OF RENAL CELL CANCER

The aim of a histopathological classification is to use morphological criteria in order to identify biologically distinct disease states, the recognition of which are of clinical value [31]. Therefore, modern tumor classifications generally are cytologically orientated, i.e. emphasizing histogenesis and differentiation. The formation of such a classification for epithelial renal tumors has been proven difficult. Previous attempts have been 'closed' systems using histology as the basis both for the classification and for the recognition of the distinct species [31].
Currently two morphological classifications are used: one according to the WHO/AFIP [2] and one according to Thoenes and Störkel [20,24]. As stated in the latter, eight different subtypes of RCA/RCC can be distinguished, related to the basic cell types of the nephron from which they are derived: (1) RCCs of the clear cell type, (2) RCAs/RCCs of the chromophilic cell type, (3) RCAs/RCCs of the chromophobic cell type, (4) RCCs of the Duct Bellini cell type, (5) RCCs of the transitional cell type, (6) RCCs of the neuroendocrine type, (7) RCAs of the oncocytic type, and (8) RCAs of the metanephroid type. These types show phenotypical/histogenetical relations to different parts or cell types, respectively, of the nephron collecting duct system [24].
Basically three growth patterns, which can be deduced from the tubule, are distinguished: (1) compact (subtype: acinar); (2) tubulopapillary, and (3) cystic. Principally, in a given tumor, all growth patterns can occur simultaneously, but generally one of them predominates. There are relationships to the cell types, although not exclusive: clear cell and chromophobe type are predominantly related to compact growth, chromophilic to tubulopapillary growth, oncocytic (true 'renal oncocytoma') is related to acinar, and Bellini duct to both compact and tubulopapillary growth.
The renal cell carcinomas are graded with respect to nuclear atypia, including the size of nucleoli, supplemented by cytoplasmic features, e.g. diminution of basic features, augmentation of eosinophilia/granularity (=mitochondria), and spindle/pleomorphic cell form. Presently, according to these parameters three grades (G1, G2, G3/4) are distinguished [20].

CYTOGENETIC AND MORPHOLOGIC CORRELATIONS

Cytogenetics allows the classification of tumors with respect to their genotypic differences. Figure 1 summarizes and correlates both morphology and cytogenetic data with respect to histogenetic aspects of most of the basic tumor subtypes mentioned above. RCCs of the transitional cell type, neuroendocrine type and RCAs of the metanephroid type are not mentioned in Figure 1 because of the limited data available.
One of the first genetic alterations in tumor development, associated with the epithelia of the proximal tubule, are trisomy 7 and loss of the Y-chromosome, probably resulting in hyperplastic and dysplastic changes.
But trisomy 7 and loss of Y may have limited or no significance with respect to RCC development and progression, because these chromosomal alterations have been demonstrated to be present in normal cells of tumor-adjacent kidney parenchyma rather than in the tumor itself [32] and trisomy 7 and trisomy 10 can be found in subpopulations of tumor-infiltrating lymphocytes [33]. Recently,

occurrence of trisomies 5, 8, and 18 has also been reported for non-neoplastic kidney tissue [34,35]. A gain of chromosome 7 may confer growth advantage to some malignant cells, because of the presence of the epidermal growth factor receptor on this chromosome [36]. The loss of one sex chromosome has been observed frequently. The non-random loss of the Y-chromosome in RCC remains obscure and is possibly age-related [37,38] The most frequent finding in RCCs of the clear cell type is a deletion or unbalanced translocation involving the short arm of chromosome 3 [23,39,40]. The breakpoints appear to cluster in region 3p11-p21, usually at 3p14. Involvement of the long arm has seldom been described. Recently, the relevant tumor suppressor gene responsible for the hereditary forms (von Hippel-Lindau disease) has been identified [41]. This gene seems also to play a role in the development of the sporadic forms, probably in combination with other gene(s) [42,43]. Moreover, a recent report suggests the presence of clear cell RCAs, showing only one deletion at 3p (3p- in Figure 1), either 3p14 or 3p25, whereas subsequent loss of the 3p21 region results in clear cell RCCs (3p= in Figure 1) [44]. Also a (partial) trisomy of chromosome 5, especially the 5q22-qter segment, is frequently found in the clear cell tumors as well as trisomy 12, and 20, loss of chromosomes 8, 9, 13, 14, and structural abnormalities of the long arm of chromosomes 6 and 10 [23,45-48], own observations).

RCAs of the chromophilic type show a typical pattern of numeric aberrations: i.e. -Y,+7,(+7),+17. Trisomy 3 is also frequently found [26,49-52]. Trisomy of chromosomes 12, 16, and 20 has been associated with the progression from the adenoma into the carcinoma stage, i.e. RCCs of the chromophilic type [50,53]. Conflicting data exist about papillary RCC showing rearrangement of the critical 3p segment, at least at a molecular level [54,55].

Several human renal cell carcinomas with X;autosome translocations have been reported in recent years (see for review [56]). The t(X;1)(p11.2;q21) appears to be a specific primary anomaly, suggesting that tumors with this translocation form a distinct subgroup of chromophilic RCC, showing clear cell features. These tumors preferentially occur in male patients [6,9,57,58], although one female case has recently been described [59].

Chromophobic carcinomas show multiple losses of entire chromosomes, i.e. loss of chromosomes 1, 2, 6, 10, 13, 17, 21, and the Y chromosome, leading to a low chromosome number. They also show quantitative as well as qualitative changes in mitochondrial DNA [60,61].

Two reports with cytogenetica data from collecting duct carcinomas revealed conflicting findings of monosomy for chromosomes 1, 6, 14, 15, and 22 in one case [62] and trisomies 7, 12, 16, 17, and 20 in the other [63]. Schönberg [64] reported involvement of the short arm of chromosome 8 related to poor prognosis and loss of the long arm of chromosome 13, both in three out of six cases.

There are no cytogenetic data on RCCs of the transitional cell type. Only one case with cytogenetics of RCC of the neuroendocrine type has been published revealing structural and numerical aberrations of chromosome 13 [65]. Molecular analysis of another case showed LOH on 3p21 [66]. Both transitional cell RCCs and neuroendocrine RCCs are not mentioned in Figure 1.

RCAs of the oncocytic type seem to be characterized by mitochondrial DNA changes [67,68], a feature they share with the chromophobic carcinomas. At least two subgroups can be distinghuished: one characterized by translocations involving 11q13 [69] and one by the combination of -Y,-1 [70,71]. Loss of chromosomes 1 and Y is also observed in chromophobic carcinomas. From a cytogenetic point of view, oncocytomas showing -Y,-1 might progress to chromophobic carcinomas through additonal chromosome losses (see Figure 1). This might explain why

oncocytomas, which are considered to be benign neoplasms, occasionally show a malignant behavior.
One case of RCA of the metanephroid type (not mentioned in Figure 1) revealed a normal (46,XY) karyotype [72].
A strong correlation between pronounced telomere shortening and the appearance of telomeric associations of chromosomes was found in three renal cell tumor subtypes (RCAs of the oncocytic type, RCAs/RCCs of the chromophobic type and RCCs of the chromophilic type), suggesting an etiological role of the loss of telomeric DNA repeats in the formation of telomeric assocations and a possible involvement of this mechanism in the pathogenesis of chromosome aberrations [73].

CYTOGENETICS AND TUMORPROGRESSION

In the clear cell RCCs, monosomy 8, 9, 13 and 14, and trisomy of chromosomes 12 and 20 seem to correlate with a higher grade, and thus progression. Also structural aberrations

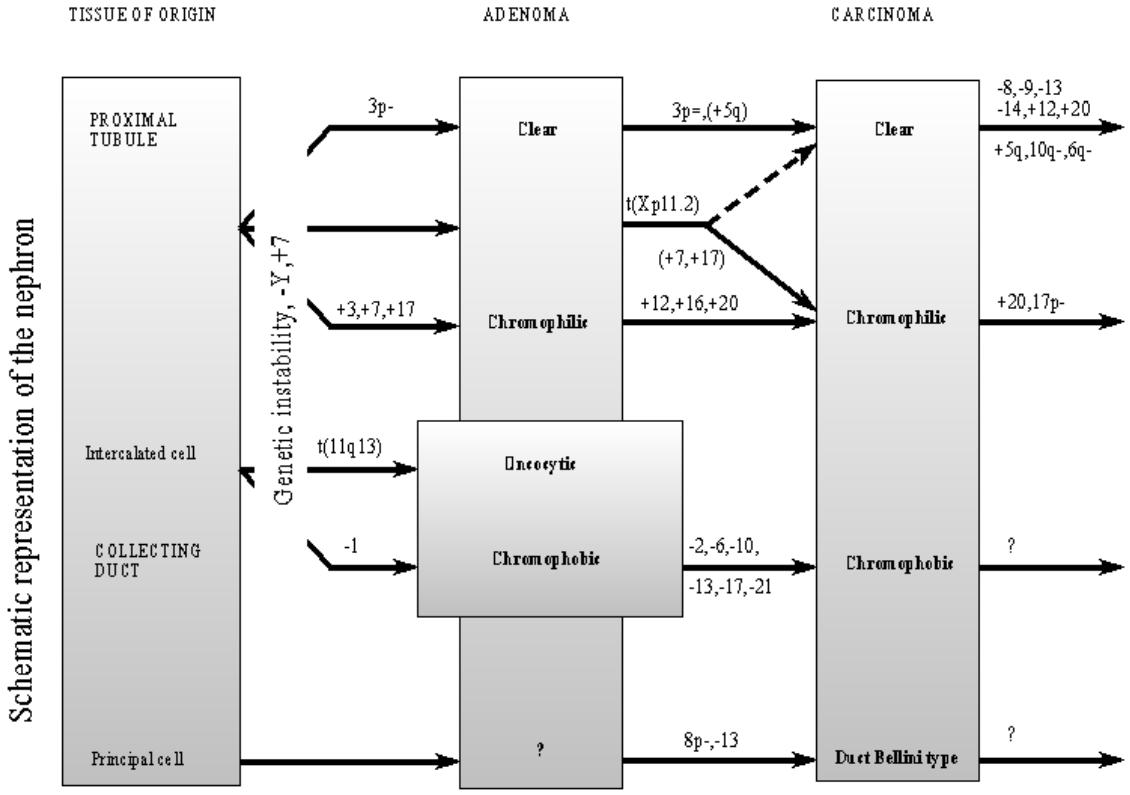

Figure 1: Proposed oncogenetic model for renal cell tumors.

(and probably loss of heterozygosity) of chromosomes 5q, 6q, 8p, 9, 10q, and 14q are associated with tumorprogression [23,74]. In RCAs/RCCs of the chromophilic type polysomies 12, 16 and 20 are associated with progression from adenoma into carcinoma stage. The loss of the extra chromosome 17(p) in RCCs of the chromophilic type tend to be related to the higher grade neoplasms, in which also a higher frequency of trisomy 20 is found [75]. It seems that tumors derived from the proximal tubule (RCAs/RCCs of the clear cell and the chromophilic cell type) share secondary karyotypic changes and this might suggest that many of the tumor suppressor loci involved may be common to the etiology of both forms [76].

Sarcomatoid transformation in RCC represents the highest form of dedifferentiation [20]. Sarcomatoid variants of RCC can in principle be deduced from all the basic cell types. Cytogenetic data on sarcomatoid RCC is scarce. Grammatico [77] reported a sole case of pleuric effusion of sarcomatoid RCC with structural abnormalities of chromosomes 1, 5, 16, and 19. Others have found a relation between p53 mutations and sarcomatoid RCC [78]. Since it is not clear which basic cell types are involved in the above mentioned cases, these data are not included in Figure 1.

CONCLUSION

Renal cell cancers (RCCs) are epithelial neoplasms that demonstrate a diversity of morphologic characteristics and clinical manifestations. The classification of the heterogeneous group of RCC is still a matter of debate. Different subtypes of renal cell carcinoma might originate from cells of the different parts of the renal tubulus.

Taken together, cytogenetic and molecular genetic studies of recent years have demonstrated that certain specific chromosomal abnormalities correlate with different histological subtypes of renal tumors. Chromosomal abnormalities are believed to be responsible for neoplastic transformation, tumor growth and tumor progression [79]. Oncogenetic studies might reveal the cell of origin, oncogenetic steps and relationship of tumors.

The view of a relation between renal cell adenomas and carcinomas is strengthened by the fact that oncocytomas and adenomas occasionally show a malignant behavior. A reasonable explanation for this exceptional behavior is that oncocytomas and adenomas probably represent the benign side of a spectrum of renal cell tumors, with renal cell carcinoma at the other extreme. If the "spectrum" concept for adenomas and carcinomas is correct, then it may be expected that there would also exist an overlap in some of the characteristics of these benign and malignant tumors. Referring to this concept, the chromophobic carcinoma could be the malignant counterpart of the oncocytoma. Both show marker proteins and ultrastructural features of the distal nephron, thus disproving the broadly accepted hypothesis that all renal cell cancers are related to the proximal tubulus.

At the (cyto)genetic level, this oncogenetic sequence might be envisaged as depicted in Figure 1.

Thus, (cyto)genetic studies of renal cell adenomas, various subtypes of carcinomas and oncocytomas, will contribute to a better understanding of the biology of these tumors, and reveal key information on the process of tumorigenesis and tumor behavior. This information may open new opportunities for (early) diagnosis and specific therapy.

CHAPTER 3

3.1 CHROMOPHILIC RENAL CELL CANCER

CHAPTER 3.1.1

CHROMOSOMAL FINDINGS AND *P53* MUTATION ANALYSIS IN CHROMOPHILIC RENAL CELL CARCINOMAS

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ABSTRACT

The chromosomal pattern of thirty-one specimens of chromophilic renal cell cancer (RCC), selected according to the criteria mentioned in the classification of Thoenes and Störkel, is presented. A high male preponderance was found (8.7:1). Cytogenetic analysis revealed a typical pattern of numeric alterations specific for this subtype in the majority of cases (i.e. -Y, +7, +12, +16, +17, and/or +20) , which is different from the chromosomal patterns found in other subtypes of RCC. Gain of chromosome 20 as well as loss of the extra copy of chromosome 17 or loss of 17p was found to be related to the higher grade chromophilic carcinomas. None of the fourteen cases examined by SSCP analysis revealed mutations of the p53 gene, indicating that other genes at 17(p) might be important in the progression of this subtype of RCC.

INTRODUCTION

The classification of the heterogeneous group of RCC is still a matter of debate. Currently two morphological classifications are used: one according to the WHO [80] and one according to Thoenes and Störkel [20,24]. As stated in the latter, eight different subtypes of RCC can be distinguished, related to the basic celltypes of the nephron from which they are derived. Attempts to correlate cytogenetic and molecular genetic data to the different subtypes of RCC mentioned in this classification have shown promising results [23,50,53]. According to Thoenes and Störkel [20,24] the cells of the proximal part of the tubule give rise to two different tumor subtypes, i.e. clear cell RCC, the most common form of RCC, and chromophilic RCC, which have a similar antigenic phenotype, but show different cytomorphological forms and growth patterns and reveal a different chromosomal pattern.

Chromophilic RCC comprises about 15% of cases, and shows a high male preponderance. These tumors, in the literature generally referred to as papillary renal cell tumors [5,81,82], usually are of

low stage and are commonly accompanied by the presence of multiple small (less than 3cm) chromophilic lesions in the adjacent non-neoplastic kidney tissue and in the contralateral kidney [5]. As a rule chromophilic RCC shows a papillary growth pattern, becoming solid in undifferentiated areas [24]. The most characteristic finding probably is the distinct chromosomal pattern, as compared to the other subtypes of RCC, although the available data is limited. Trisomy of chromosomes 7 and 17 combined with loss of the Y chromosome is a frequent finding and is also observed in adenomas of the chromophilic type [49,50]. Trisomy of chromosomes 12, 16, and 20 has been associated with the progression from adenomas into the carcinoma stage [50,53].
In order to gain more insight in the process of development and progression in this subtype of RCC, we examined the chromosomal patterns of thirthy-one specimens of chromophilic RCC from twenty-nine patients. In addition mutation analysis for the *p53* gene was performed using the SSCP method. An attempt is made to find a relation between tumorprogression within this subtype and the observed changes.

MATERIALS AND METHODS

Fresh representative samples of all tumors were submitted for cytogenetic investigation. The tumors were classified according to Thoenes et al [20]. A picture of one of the cases (case 92) is given in Figure 1. Part of the tissue was cultured for 5-7 days in RPMI 1640 supplemented with FCS (16%), glutamine and antibiotics. The cultures were harvested and chromosome preparations were made according to standard cytogenetic techniques. The chromosomes were G-banded using trypsin or pancreatin and karyotypes were described according to the ISCN'95 guidelines for cancer cytogenetics.

Screening for p53 mutations
In a first approach we carried out a single strand conformation polymorphism analysis (SSCP). Genomic DNA from frozen tissue was isolated according to standard protocols. Paraffin embedded tissue sections were deparaffinized and genomic DNA was extracted in with a chelating ion exchange resin, chelex^{R} 100 (Biorad, Richmond, CA) a one-step DNA extraction protocol. To amplify p53 genomic DNA isolated from frozen tissue a radioactive PCR was carried out using primers previously described [83]. When the resulting PCR product exceeded 250 bp, 10 units of an appropiate restriction enzyme were added directly to the PCR mixture, followed by an incubation at 37^{o}C for 2 hours. Since it is difficult to amplify long DNA fragments from paraffin material we selected new primer pairs revealing PCR products of approximately 200 bp or less for amplification of DNA from paraffin embedded tissue sections. The primer sequences used are for exon 2 p53-2F, 5'-ttggaagcgtctcatgctgg-3' and p53-2R 5'ctgcccttccaatggatcc-3'; for exon 3 p53-3F, 5'-cctagcagagacctgtggg and p53-3R 5'-gagcagtcagaggaccagg-3'; for exon 4 p53-4AF 5'-ggacctggtcctctgactg-3' and p53-4AR 5'-gtgtaggagctgctggtgc-3'; p53-4BF 5'-agctcccagaatgccagagg-3' and p53-4BR 5'-cagggcaactgaccgtgcaag-3'; for exon 5 p53-5F 5'-ttcctcttcctacagtactcc-3' and p53-5R 5'-ccagccctgtcgtctctcc-3'; for exon 6 p53-6F 5'-cact-gattgctcttaggtctg-3' and p53-6R 5'-actgacaaccacccttaacc-3'; for exon 7 p53-7F 5'-gcactggcctcatcttggg-3' and p53-7R 5'-gcacagcaggccagtgtgc-3'; for exon 8 p53-8F 5'-cctatcctgagtagtggtaa-3' and p53-8R 5'-tccaccgcttcttgtcctgc-3'; for exon 9 p53-9F 5'-tat-

caccttttccttgcctc-3' and p53-9R 5'-ccaagacttagtacctgaag-3'; the primers for the amplification of exon 10 and 11 are the same as used for the genomic DNA isolated from frozen tissue. The paraffin material was amplified in two PCR reactions, first a non radioactive PCR reaction for 30 cycles, second, a radioactive PCR in which 1 µl of the first PCR mixture was amplified in 30 cycles. SSCP analysis was performed as described previously [84].

RESULTS

The cytogenetic results and patient data of thirty-one chromophilic RCC specimen are presented in Table I. Only three tumors occurred in female patients, giving in this study a male to female ratio of approx. 8.7:1. In two of the patients two RCCs were excised. Patient 3187 had one grade I and one grade II chromophilic RCC, the cytogenetics of which have been published [50]. Patient 3862 presented with two grade I chromophilic tumors. Case 2405 was published before as being one of the rare chromophilic RCCs carrying a X;autosome translocation. However, molecular analysis revealed that the breakpoint in Xp11.2 differed from the other RCCs with X;autosomal translocations [56].
The number of chromosomes of all cases varied between 40 and 53. Loss of the Y chromosome was found in twenty-three out of twenty-seven succesfully analyzed male tumors (85%). With respect to the grade I carcinomas, eight out of nine (one was a failure) male cases revealed loss of the Y chromosome (89%) and all nine had a trisomy 17 (100%). Two cases had a trisomy 20 (22%). Seven grade I tumors showed clonal structural abnormalities (78%).
Seventeen grade II carcinomas were analyzed. Twelve out of fourteen grade II male cases revealed loss of the Y chromosome (87%). Ten cases had a trisomy 17 (59%), and seven cases showed a trisomy 20 (41%). Clonal structural abnormalities were found in nine tumors (53%). Two of the grade II tumors had a structural rearrangement of chromosome 17 resulting in loss of part of 17p. The karyotype of one specimen of grade II chromophilic RCC (case 92) is given in Figure 2.
Only one out of four grade III chromophilic carcinomas had a trisomy 17 (25%), and one had clonal structural chromosomal abnormalities. No trisomy 20 was observed. Trisomy 7 was the only abnormality shared by these four cases.
SSCP analysis was performed for the *p53* gene on 14 cases of the present study, including five grade I RCCs (cases 1, 79, 9034,11137 and 48), six grade II RCCs (cases 11, 92, 5561, 11843, 1422, and 2730), and three grade III RCCs (cases 12013, 11834 and 50). No abnormal patterns were observed.

DISCUSSION

In order to further unravel the oncogenesis and progression of the different subtypes of RCC, we cytogenetically examined thirty-one chromophilic carcinomas from twenty-nine patients. The male to female ratio was 8.7:1, which is higher than in the cases described by Kovacs et al. [82].

Table I: Cytogenetic and patient data of all chromophilic renal cell carcinomas, used in this study.

Pat. nr.	Sex	Age	Grade	Cytogenetics
00001	M	51	G1	46-52,X,-Y,+3,del(6)(q22),+7,+12,+16,+17,add(19)(p13),+20 [cp5]
00079	M	44	G1	45-47,XY,+17,idic(21)(p13) [cp10]
09034	M	78	G1	50,X,-Y,+7,+7,der(12)t(3;12)(q11;p13),+16,+17,+20 [cp9]
02405	M	77	G1	49-53,-Y,t(X;10)(p11.2;q23),+3,+5,+7,+7,+12,+16,+17 [cp9]
11137	M	57	G1	Failure
00048	M	71	G1	46-50,X,-Y,add(1)(p13),+3,+7,der(10)t(1;10)(p13;p15),+16,+17,+21 [cp7]
03187 II	M	60	G1	48,X,-Y,+7,+16,+17 [cp20]
03862 I	M	61	G1	49,X,-Y,+7,+7,+12,+17 [cp7]
03862 II	M	61	G1	47,X,-Y,t(5;6)(q23;p13),+7,+17 [cp5]
03620	M	62	G1	50,X,-Y,t(5;18)(q23;p13)+7,+7,+16,+17 [cp11]
00011	M	63	G2	46-48,X,-Y,+7,-9,del(11)(q21 q24),+12,der(21)t(17;21)(q11.2,p13),+r [cp11]
00092	M	59	G2	48-49,X,-Y,add(7)(q31),+add(7)(q31),+12,+13,+17 [cp10]
05561	M	42	G2	42-47,X,-Y,+7 [cp5]
11843	M	43	G2	39-40,X,-Y,dic(1;2;22)(1qter->1q11::2q37->2p11.2::22p11->22qter),der(8)t(1;2;8)(q24;q21 q37;q11),add(13)(p11),add(15)(q25),-16,+17,-18,-19,-21 [cp9]
01422	M	77	G2	43-48,X,-Y,der(6)t(6;?;6)(p11.2;?;q15),+7,+der(7)t(3;7)(q13.3;q36),del(11)(q21),der(17)t(1;17)(p22;q25) [cp9]
02730	M	41	G2	43-48,X,-Y,t(1;1)(p32;q21),+12,add(12)(q24),-14,+1-2mar [cp11]
03187 I	M	60	G2	52,XY,+3,+7,+12,+16,+17,+20 [cp18]
03634 I	M	81	G2	51,X,-Y,+3,+7,+12,+16,+17,+22 [cp14]
03234	F	67	G2	49,X,-X,add(2)(pter),+7,+16,+17,+20 [cp18]
03573	M	41	G2	52,X,-Y,add(2)(pter)+3,+6,+7,+7,+12,+17,+20 [cp19]
02505	M		G2	46,XY [10]
03098	F	53	G2	50,XX,+7,+12,+16,+17 [cp22]
04129	M	51	G2	50,X,-Y,+3,+7,+16,+17,+20 [cp15]
04180	M	70	G2	50,X,-Y,+3,+7,+16,+17,+20 [cp11]
04382	M	67	G2	49,X,-Y,+3,+7,+12,+16,-21 [cp9]
04415	M	54	G2	49-51,X,-Y,+3,+7,+12,+16,+17,+20,+21,+mar [cp8]
04469	F	69	G2	50-51,X,-X,+5,+7,+12,t(14;22)(q10;q10),+16,+17,+20 [cp15]
03752	M	80	G3	49,X,-Y,del(2)(q21),+3,+7,+10,add(12)(pter),+17 [cp13]
12013	M	74	G3	45-48,X,-Y,+7,+10 [cp12]
11834	M	64	G3	45-48,XY,+7 [cp5]
00050	M	60	G3	47,X,-Y,+7,+18 [cp10]

Loss of the Y chromosome was found in 85% of male cases, resembling the findings by others [50,53,82]. The high incidence of Y chromosome loss in this subtype, combined with the strong male preponderance, suggests that loss of specific sequences harboured on the Y chromosome might be important in the development of this subtype. Kovacs et al. [82] postulated that a tumor suppressor gene localized at one of the homologous regions of the X and Y chromosomes might play a role in the oncogenesis of these neoplasms. Interestingly a probable variant of chromophilic RCC carrying abnormalities involving Xp11.2, also developing predominantly in males, tend to retain their Y chromosome [56,58].

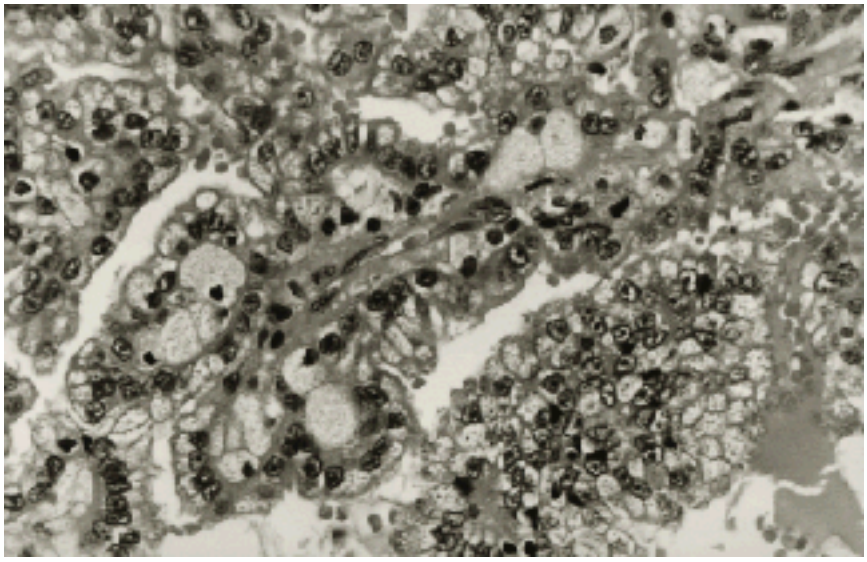

Figure 1: Histology of one of the grade II chromophilic RCCs (case 92) (HEx341)

Overall gain of chromosome 17 is found in 90% of the chromophilic neoplasms of the present study. All of the grade I carcinomas have a trisomy 17. In the grade II tumors 59% and in the grade III tumors only 25% have this specific abberration. These results indicate that progression of the chromophilic carcinomas is associated with loss of the extra copy of chromosome 17. Of the cases published by Kovacs et al.[53] similar findings are observed. They found a trisomy 17 in all adenomas, 80% grade I carcinomas, and 50% grade II carcinomas. Furthermore their only grade III carcinoma lacked the trisomy 17. In addition some of the chromophilic carcinomas revealed structural abnormalities involving chromosome 17 ([47,53], present findings). Two of the grade II chromophilic carcinomas of the present study have a structural aberration resulting in loss of part of 17p. Loss of sequences at chromosome 17p have been associated with tumorprogression in RCC. Reiter et al. [85] found LOH of 17p in 48% of RCC cell lines, which were all derived from patients with advanced (Robson stage IV) disease. Presti et al [46] described 17p deletions predominantly occurring in high stage tumors. Furthermore both Presti and Ogawa [46,86]

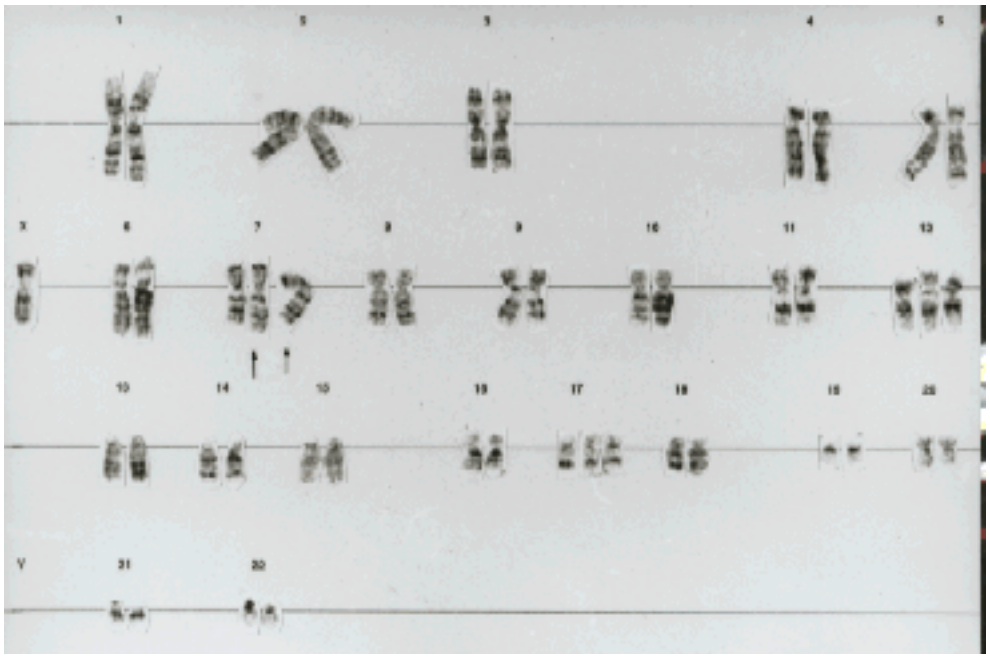

Figure 2: Karyotype of case 92, revealing a 48,X,-Y,add(7)(q31),+add(7)(q31), +12,+17 chromosomal pattern.

demonstrated that the papillary type of RCC show a relatively frequent loss of 17p compared to clear cell RCC. Mutations of the *p53* tumor suppressor gene, assigned to 17p, are observed in a variety of human tumors. This gene might also be important in the progression of chromophilic RCC. In this light we performed PCR-SSCP analysis on fourteen cases of the present study for mutations in the *p53* gene. No mutations were found in the coding region of this gene, suggesting that *p53* does not play a role in the progression of chromophilic RCC. Similar findings were observed by Uchida et al [87]. The authors observed LOH at 17p in 6 out of 29 cases, but did not find mutations in the *p53* gene. Our findings, together with those mentioned in the literature, indicate that, although the *p53* gene does not seem to be involved, a relation exists between loss of 17p sequences and tumorprogression. Further studies should learn whether other genes at 17p are important in this matter.

It is true that RCC of the clear cell type predominantly has a compact growth pattern, whereas chromophilic RCC mainly grows papillary. This relation is however not an exclusive one. RCC with 3p deletions can be highly papillary and predominantly granular, and chromophilic RCC with a trisomy 17, can reveal a compact growth pattern and mimick clear cell tumors, due to the accumulation of fat in their cytoplasm [20]. One of the chromophilic carcinomas (Table I, case 11) has a compact growth pattern. The chromosomal pattern of this tumor shows extra copies of chromosome 7 and the long arm of chromosome 17, and loss of the Y chromosome. Furthermore no deletions of the short arm of chromosome 3 were observed. The distinct chromosomal patterns of the two subtypes of RCC are therefore probably not related to the papillary growth pattern but

to the specific cell type of this neoplasm, illustrating the importance of recognizing the basic cell type in RCC and the value of a classification based on the different cell types observed in these neoplasms.
Trisomy of chromosome 20 is thought to play a role in the transition from adenoma into carcinoma. Our results indicate that gain of this chromosome also reflects progression within the chromophilic carcinoma. Only two out of nine grade I carcinomas show trisomy 20 (22%), whereas seven out of seventeen grade II tumors have an extra copy of this chromosome (41%). Of interest is patient 3187 which had one grade I and one grade II chromophilic carcinoma and reveals the trisomy 20 only in the latter. The absence of trisomy 20 in our grade III carcinomas might be explained by the fact that in three of the four cases we only found abnormalities also observed in non neoplastic kidney tissue [35], and probably failed to grow tumorcells. Our results suggest that gain of chromosome 20 not only marks the transition from adenoma to carcinoma, but also reflects progression within the chromophilic carcinomas.
In conclusion: Chromophilic RCC has a chromosomal pattern different from those found in other RCC subtypes. A classification based on the specific cell types found in the nephron, reveals a more exclusive relation between chromosomal pattern and RCC subtype. Specific for chromophilic RCC is gain of chromosomes 7 and 17 and loss of the Y chromosome, events that occur early in the development of these tumors. The high male preponderance and the frequent Y chromosome loss indicates that sequences harboured at the Y chromosome might be especially important in the oncogenesis of chromophilic RCC. Tumorprogression within the chromophilic carcinomas seems to be reflected by gain of chromosome 20 and loss of 17(p). No mutations were detected for the *p53* gene, suggesting that *p53* most likely does not play an important role in the progression of chromophilic RCC. Whether or not other genes at 17p are involved has to be elucidated. In order to unravel the oncogenesis and relationship of the different subtypes of RCC, expanding the cytogenetic and molecular data on the subtypes other than clear cell RCC are of the utmost importance.

CHAPTER 3.1.2

TRISOMY 17 IN MULTIPLE CHROMOPHILIC/PAPILLARY RENAL CELL CANCER: KEY ALTERATION OR SECONDARY CHANGE?

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ABSTRACT

Chromophilic or papillary renal cell cancer is a distinct subtype of renal tumors, which frequently shows a multiple appearance in patients with and without a family history of renal cancer. Genetically these tumors are characterized by a combination of autosomal trisomies, with trisomy 17 as the most consistent one. We present a cytogenetic analysis of multiple tumors of two patients with multiple chromophilic/papillary RCC, one sporadic and one known to have at least three family members affected with this disease. The different tumors of both patients revealed the characteristic autosomal trisomies associated with the chromophilic/papillary subtype and trisomy 17 was observed in all cytogenetically examined tumors. Previously we have shown that in sporadic multiple chromophilic RCC different tumors of the same patient duplicated the same parental chromosome 17 allele, whereas different chromosome 7 alleles were involved. Chromosome 7 and 17 allelic imbalance analysis, performed on different tumors of the familial case, revealed imbalance of the same parental chromosome 7 allele in five of six tumors. Surprisingly, chromosome 17 allelic imbalance, observed in all six tumors, showed random duplication of the chromosome 17 alleles. Therefore, chromosome 17 most likely does not carry the putative inherited mutation responsible for the predisposition to develop familial chromophilic tumors and should be considered an early secondary genetic change, rather than the initiating event. Whether or not trisomy 17 initiates tumor formation in sporadic cases is not clear, since not all examined tumors of the sporadic cases showed allelic imbalance for chromosome 17.

INTRODUCTION.

Renal cell cancer (RCC) comprises a heterogeneous group of tumors. A recent morphological classification divides RCC into distinct subtypes according to their presumed cell of origin in the mature renal tubular system [20,24]. Clear cell (non papillary) and chromophilic (papillary) RCC

account together for approximately 90% of all RCC. Although both have a similar antigenic phenotype, their genetic constitution is distinct. Deletions of the short arm of chromosome 3 and mutations of the *VHL* gene are characteristic findings in clear cell RCC, whereas chromophilic or papillary RCC shows a unique combination of numeric aberrations; i.e. -Y,+3q,+7,+12,+16,+17,+20, but no mutations of the VHL gene [24,26,40,53,75]. There is growing evidence that papillary RCC has a genetically defined adenoma stage, characterized by a -Y,+7,+17 chromosomal pattern. Subsequent gain of chromosome 12, 16, and/or 20 is associated with progression to a carcinoma [26,49,50,53].
The presence of multiple and/or bilateral tumors is indicative for hereditary and familial forms of RCC. Hereditary forms of clear cell RCC, arising in patients with constitutional balanced translocations involving chromosome 3, or in *VHL*-related families have been extensively studied [88]. Familial cases of papillary RCC have been described, but the genetic defect reponsible for the predisposition to develop multiple tumors in these families is not yet known [89-91]. Linkage analysis indicated that the disorder could not be linked to polymorphic markers of chromosome 3p [89]. Cytogenetic analysis of different papillary tumors of two affected members of another family showed the autosomal changes, including trisomy 17, frequently associated with this RCC subtype. The adjacent non-neoplastic kidney tissue revealed a fully normal karyotype [90].

Multiple and bilateral papillary tumors are also found in patients without a family history of RCC [50,53]. A high incidence of papillary tumors has been observed in the kidney parenchyma of patients with sporadic (non familial) papillary RCC. In contrast, in kidneys of patients with non papillary cell RCC less tumors were observed [92,93]. Genetic changes observed in sporadic multiple papillary RCC resemble those of solitary cases [50,53].

In an attempt to unravel the development of familial and sporadic cases of multiple chromophilic/papillary RCC, we cytogenetically examined a number of different tumors arising in two patients, one of which had a family history of RCC. Chromosome 7 and 17 imbalance analysis was performed on different tumors of the familial case. Results of karyotyping and chromosome 7 and 17 imbalance studies are compared with similar cases in the literature.

MATERIALS AND METHODS

SPORADIC CASES

Case 1 and case 2:
The patient data of these two cases have been published elsewhere ([44] case 3 and 2 respectively).

FAMILIAL CASE

Case 3:
A 32-year old male presented with multiple neoplastic lesions in the left kidney. A grade I carcinoma measuring 15 x 6 x 12 cm was excised. The tumor was confined to the kidney and the weakly eosinophilic cells showed a tubulo papillary growth pattern. Interstitial foam cells were present and a fibrous capsule surrounded the tumor. In addition, multiple small lesions of approx. 1 mm were removed from the adjacent kidney tissue. All lesions were of the chromophilic type. The right kidney of this patient was removed four years earlier, because of the presence of two chromophilic carcinomas. In the nephrectomy specimen also multiple adenomas were encountered,

showing the same histologic picture as the present lesions. Furthermore the patient was known to have at least three family members from three different generations affected with multiple chromophilic RCC.

METHODS

Fresh representative samples of normal and tumor tissue of case 1 and case 3 were submitted for cytogenetic analysis. The tissues were enzymatically disaggregated using collagenase type II, and cultured for 7-14 days in RPMI 1640 supplemented with FCS, glutamine and antibiotics.

Table I: Cytogenetic data of cases 1 and 3

CASE	TISSUE	CYTOGENETICS
Case 1	Kidney tissue	47,XY,+7[cp3]/46,XY[7]
	Adenomas (1+2)*	47,X,-Y,+7,+17[4]/47,X,-Y,der(1) t(1;3)(q44;q11),+7,+17[5]
	Carcinoma (G2)	47~50,X,-Y,+2,+7,+7,-9,der(9) t(9;17)(q34;q11),der(9)t(9;17) (q34;q21),der(9)t(9;?;17) (q34;?;q21),+12,-14,+17, der(17)t(17;18)(p11.2;q11.2), der(17)t(7;17)(p13;p12),-18, +20,-21[cp21]
Case 3	Blood	46,XY[10]
	Kidney tissue	46,XY[10]
	Adenoma 1	46~47,X,-Y,+7,+17[cp6]/47,idem, der(2)t(2;3)(p25;q11)[cp6]
	Adenoma 2	47~48,XY,+12,+17[cp6]/48,XY, der(2)t(2;?;3)(p21;?;q11)+12, add(14)(p11),+17[cp4]
	Adenoma 3	47,X,-Y,+7,+17[10]
	Adenoma 4	47,X,-Y,+7,+12,+17,-21[7]/47, idem,add(22)(p13)[3]
	Adenoma 5	49~50,XY,+del(3)(p11),+7,+16, +17,+20[cp3]/48,XY,+7,+17[8]
	Carcinoma (G1)	45~47,X,-Y,+7,+16,+17,-21[cp9]

The cultures were harvested and chromosome preparations were made according to standard cytogenetic techniques. Karyotypes were described according to the ISCN'95 guidelines.

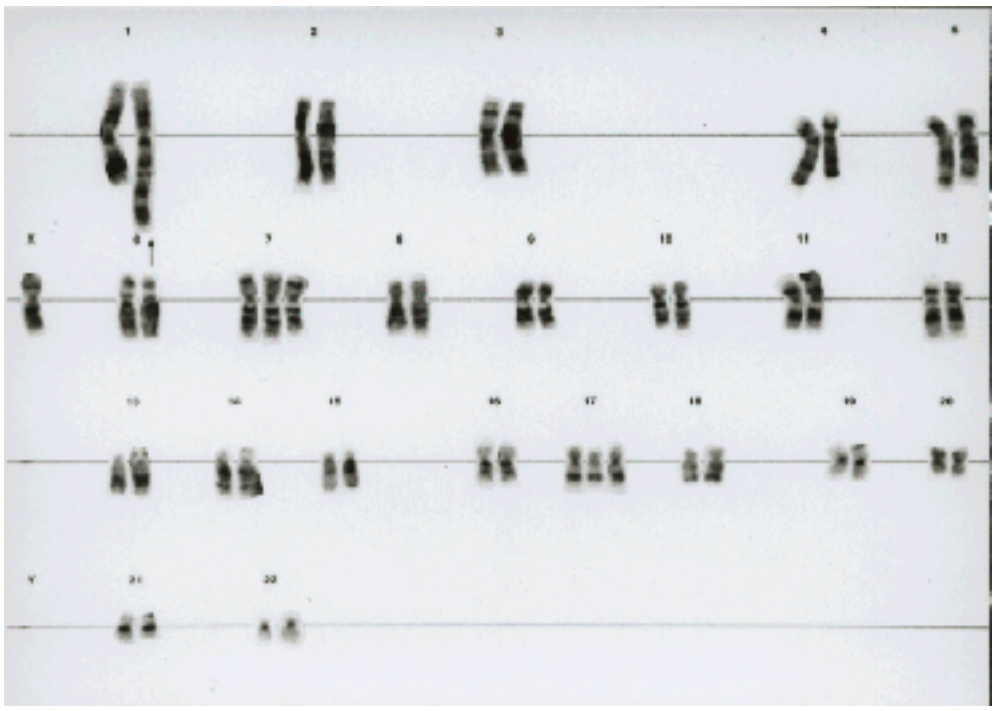

Figure 1A: Representative karyotype of the adenomas of case 1

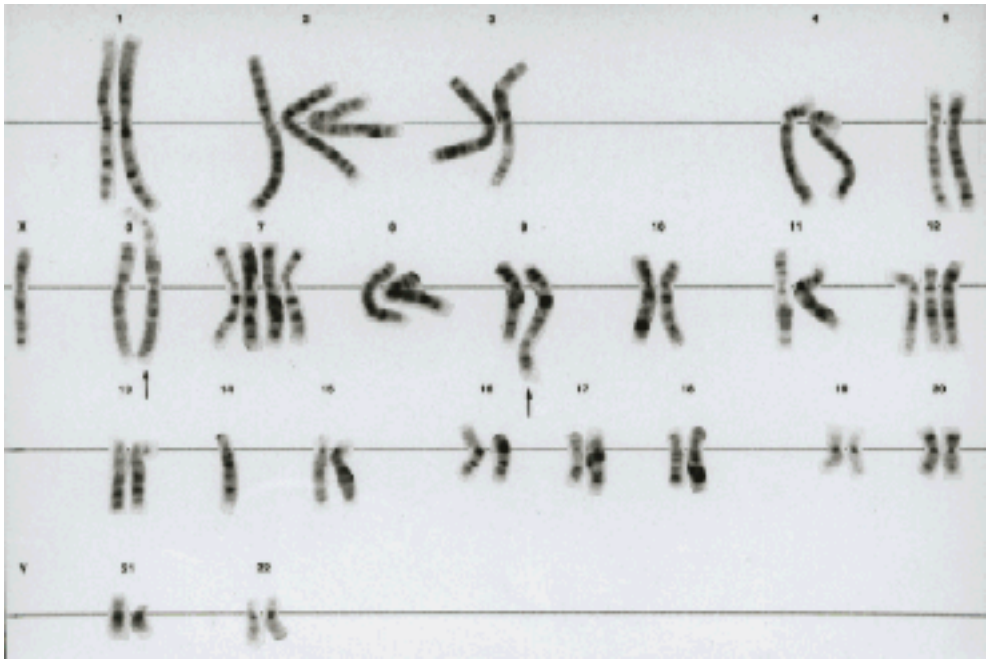

Figure 1B: Representative karyotype of the grade II carcinoma of case 1

Different tumor samples of case 3 were examined for chromosome 7 and 17 allelic imbalance. Chromosome 7 and 17 imbalance analysis of cases 1 and 2 has been described by van den Berg et al. [44]. Of case 3, the different tumors were analyzed with D7S471 and D7S473 for chromosome 7 imbalance, and with D17S520 and D17S514 for chromosome 17 imbalance. Analysis of allelic imbalances were carried out as described previously [94]. Primer sequences and PCR conditions were obtained from the Genome Data Base at Johns Hopkins University in Baltimore. Single Strand Conformation Polymorphism (SSCP) analysis of the VHL gene was performed on normal and tumor DNA of all three cases as described previously [95].

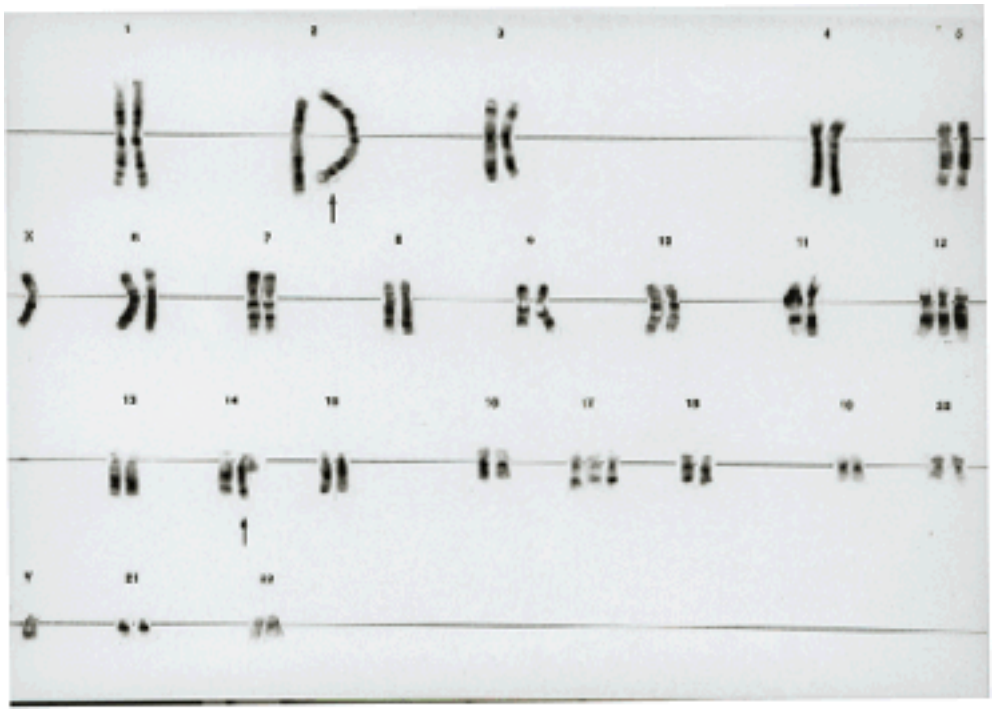

Figure 1C: Representative karyotype of adenoma 2 of case 3

RESULTS

The constitutional karyotypes of patients 1 and 3 were normal. The results of the cytogenetic analysis of the different tumors of these patients are given in Table I. Unfortunately from the second case neither constitutive nor tumor tissue was available. From the adenomas of case 1 two clones were found, one with a 47,X,-Y,+7,+17[4], the other with a 47,X,-Y,der(1)t(1;3)(q44;q11),+7,+17[5] chromosomal pattern (Fig 1A). Since the two adenomas had by accident been cultured together, we cannot tell whether these different clones represent only one or both of the adenomas. The grade II chromophilic carcinoma showed complex rearrangements. In addition to numeric changes, translocations preferentially involving one of the three copies of

chromosome 17 were observed (Fig 1B). Furthermore, several telomeric associations were found. The adjacent apparantly normal kidney tissue of this patient revealed gain of chromosome 7. Five adenomas and the grade I carcinoma of case 3 showed the autosomal changes specific for the chromophilic subtype. All had a trisomy 17, three of them a gain of the long arm of chromosome 3, more precize, the region 3q11-3qter (Fig 1C). Normal kidney tissue of this patient had a normal karyotype.

Table II: Results of chromosomes 7 and 17 allelic imbalance analysis of all three cases. Alleles are numbered a and b and represent either maternal or paternal inherited alleles.

	Case Nr (n)[1]	Chromosome 7 allele		Chromosome 17 allele	
		a	b	a	b
Sporadic[2]	Case 1 (5)	2	0	3	0
	Case 2 (9)	2	5	8	0
Familial	Case 3 (6)	5	0	3	3

[1] Number of examined tumors per case
[2] These cases have been described previously [44]

The results of chromosome 7 and 17 allelic imbalance studies of all three cases are compiled in table II. Allelic imbalance analysis of five adenomas and of the grade I carcinoma of case 3 (the familial case) showed chromosome 7 allelic imbalance in 5 tumors (Fig 2). In the remaining adenoma (A2) the ratio was 0.9, which is defined as an ambiguous result [94]. In all tumors, the same parental chromosome 7 allele was involved. Chromosome 17 allelic imbalance was observed in all six tumors involving different parental alleles. As can be seen by the ratios shown in Figure 2, each allele appeared to be duplicated in three tumors. A screening for mutations of the *VHL*-gene by SSCP analysis did not reveal any aberrant pattern for normal tissue of the three patients, thus making presence of *VHL*-related disease unlikely.

DISCUSSION

Using a cytogenetic approach no differences were found between the sporadic case and the familial case. The observed chromosomal patterns resembled those of solitary as well as multiple chromophilic/papillary RCC described in the literature, implying that solitary, multiple and familial papillary RCC develop through similar genetic events [26,40,50,53,75]. In the present study, the different tumors of case 1 share three numeric changes, i.e., -Y, +7, +17, and in case 3 all tumors have trisomy 17, suggesting a significant role for trisomy 17 in these neoplasms. Hence trisomy 17 may be an early oncogenetic step in the development of sporadic and familial tumors.

Allelic imbalance analysis of chromosomes 7 and 17 in patients with sporadic multiple chromophilic/papillary RCC (table II, case 1 and 2) showed involvement of different chromosome 7

parental alleles in different tumors, but per patient the same chromosome 17 parental alleles were involved [44]. Similar findings, concerning chromosome 17, were observed in two cases of sporadic multiple papillary RCC published by Kovacs

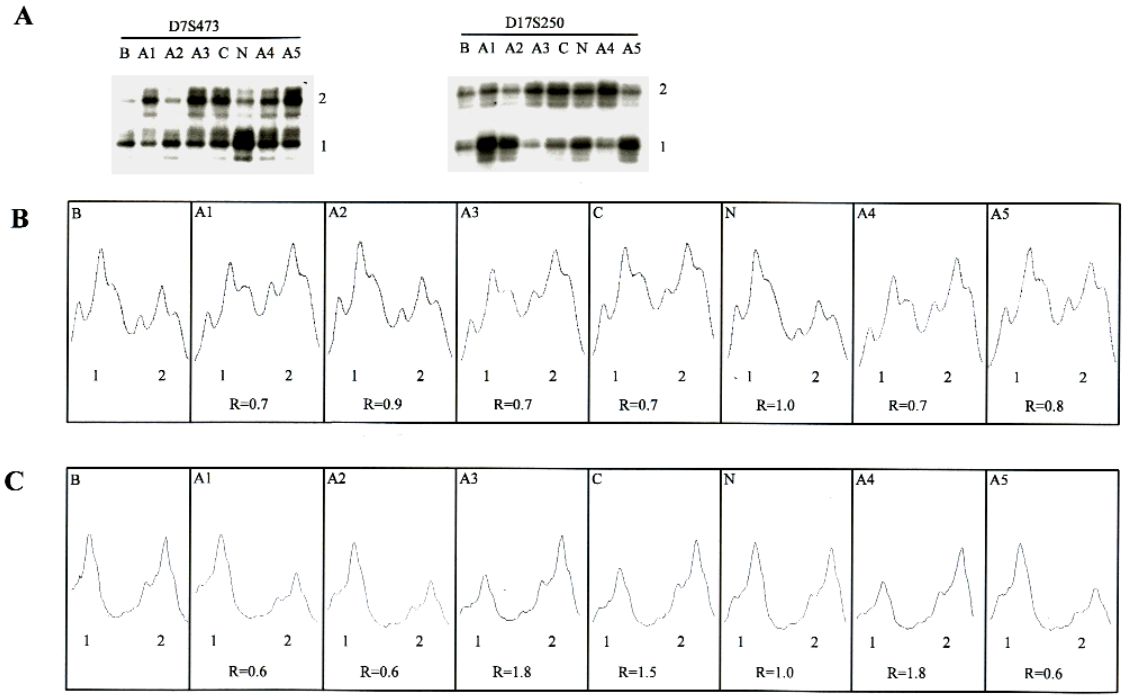

Figure 2: Analysis of allelic imbalances for chromosomes 7 and 17 for case 3.
A: Autoradiographs
B: Results of densitometric scanning of the autoradiograph obtained for D7S473
C: Result of densitometric scanning of the autoradiograph obtained for D17S250
N=normal kidney tissue; B=blood; C=carcinoma; A1-5=adenomas; R=relative signal intensity ratio (=quotient of the signal intensity ratio for the alleles in normal tissue and the signal intensity ratio for the alleles in the tumor) [94]

[26], although one of these patients had bilateral tumors and showed involvement of one allele in the different tumors of one kidney, and of the other allele in the different tumors of the other kidney. These findings indicate that in sporadic cases of multiple chromophilic or papillary RCC, the different tumors, at least those of the same kidney, most likely have a monoclonal origin and trisomy 17 must be an early step in their development. Since most of the tumors are adenomas, which have no metastatic potential, their monoclonal origin cannot be explained by metastatic disease. Persisting embryonic rests have been suggested to be the precursor lesions of papillary RCC [26]. In our view, it might well be that the first oncogenetic step in the development of sporadic tumors takes place during kidney development. One of the cells of the metanephros, the tissue from which the proximal and distal tubules originate, might gain an extra chromosome 17

due to a mitotic error. This cell continues to proliferate and differentiate in a normal way until kidney development is completed, resulting in a number of tubules containing cells, which have the same extra chromosome 17. Additional changes, such as -Y, +3q, +7, +12, +16, and/or +20 would then be necessary for development into chromophilic/papillary adenomas and carcinomas. Such an accumulation of changes might take most part of human life, implying a low proliferative potential of the precursor lesions. This is also reflected by the fact that a high number of chromophilic tumors are adenomas or low-grade carcinomas, showing no or a low metastatic potential, respectively [5,16]. The above proposed oncogenetic pathway, suggesting trisomy 17 to be an embryonal event, might explain the monoclonality of multiple, mostly benign, tumors. However, since not all examined tumors revealed allelic imbalance for chromosome 17 [44], it remains to be established whether trisomy 17 initiates tumor formation in these patients.

Allelic imbalance analysis performed on different tumors of the familial case, revealed a clear chromosome 7 allelic imbalance in five of six tumors. The same parental chromosome 7 allele appeared to be duplicated in these tumors. Chromosome 17 allelic imbalance was found in all six tumors. Surprisingly, different parental alleles were involved. Each of the different chromosome 17 alleles appeared to be duplicated in three tumors (Table II). It has been suggested that a somatic mutation or imprinting of chromosome 17 is the initiating event in the development of papillary RCC [26]. Trisomy of specific chromosomes is a common genetic change in experimental tumors of transformed rodents, as has been described [26]. In such cases preferential duplication of chromosomes carying the genetic mutation is found [96,97]. If chromosome 17 harbors a gene whose mutation is responsible for the predisposition to develop chromophilic/papillary tumors in these families, one would expect that in familial cases the same parental allele, namely the inherited one carrying the mutation, should be duplicated. In contrast, we find that both alleles are randomly involved in the process. These findings suggest that chromosome 17 does not carry the putative mutation, predisposing to chromophilic/papillary RCC in this family. We have no clue as to which inherited defect is responsible for the disease, nor do we know its localization, but most likely it is also not linked to chromosome 3 [89].

Based on the above discussed genetic findings, we propose that in familial multiple chromophilic/papillary RCC, trisomy 17 is not the first genetic change, nor does chromosome 17 carry the putative inherited mutation responsible for the predisposition to develop these neoplasms. Which genetic change precedes trisomy 17 in familial tumors is presently unknown, but most likely it is not linked to chromosome 17, nor to chromosome 3. Sporadic cases may well have an embryonal origin, and trisomy 17 may be an early step in their development. Since not all examined tumors exhibit chromosome 17 imbalance it remains to be established whether or not trisomy 17 initiates tumor formation in sporadic cases. Of course, this suggestion will remain speculative, until the presumed specific gene(s) responsible for the development of solitary, multiple and familial cases of chromophilic RCC are identified.

CHAPTER 3.1.3

GENETICS AS A DIAGNOSTIC TOOL IN SARCOMATOID RENAL CELL CANCER

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ABSTRACT

Renal cell cancer comprises a heterogeneous group of tumors, which currently can be subdivided into morphologically distinct entities, each characterized by a specific combination of genetic changes. Sarcomatoid transformation might occur in any of the subtypes, resulting in tumors consisting of both carcinomatous and sarcomatous components. The specific diagnosis of these neoplasms, as to tumor subtype, is usually made on the histologic properties of the carcinomatous tissue present. However, this might not reflect the true nature of the sarcomatous component. Since the genetic changes associated with the development of the different subtypes of renal cell cancer are well established, this knowledge might serve as a tool in diagnosing sarcomatoid tumors. Assessing the genetic constitution of the latter may lead to a correct diagnosis. It may also provide valuable information about the genetic changes associated with sarcomatoid transformation. Hence, we performed a genetic characterization of a case of sarcomatoid renal cell cancer, histologically diagnosed as being of the chromophilic type. The observed genetic changes included loss of 3p, 6q, 8p, 9, 13, 14, 17p, and gain of 5, 12, and 20, and a mutation in the coding region of the p53 gene. This combination of genetic changes point to a clear cell rather than a chromophilic origin of the sarcomatoid tumor investigated, indicating that the genetic constitution of sarcomatoid tumors may be a more reliable indicator of tumor subtype than histologic appearance.

INTRODUCTION

Sarcomatoid transformation is a unique type of tumor progression in renal cell cancer (RCC) and occurs in 1 to 1.5% of cases [5]. Sarcomatoid RCC has a poor prognosis. These neoplasms are usually large invasive tumors and many cases have already been widely disseminated at diagnosis.

Histologically, sarcomatoid RCC consists of both carcinomatous and sarcomatous components, the latter being characterized by a spindle cell pattern with little differentiation. Therefore, the diagnosis is usually made on the properties of the carcinomatous tissue, assuming that it will be representative for the sarcomatoid component as well. When the entire neoplasm has a sarcomatous histologically dedifferentiated appearance, a correct diagnosis is difficult to make.

The morphological classification of Thoenes and Störkel [20,24,98] divides RCC into different subtypes based on morphologic, histochemical, and electron-microscope data. Evidence is accumulating that each of the different subtypes has a distinct somatic-genetic constitution [24,28]. Clear cell carcinoma is characterized by 3p deletions, chromophilic or papillary RCC shows a specific combination of autosomal trisomies, i.e., +3q, +7, +12, +16, +17, +20. The chromophobe subtype has a low chromosome number, revealing mostly loss of chromosomes 1, 2, 6, 10, 13, 17, and 21. Oncocytomas have either translocations involving 11q13 or show loss of chromosomes 1 and Y. Sarcomatoid transformation seems to occur in most of the RCC subtypes mentioned in this classification [20]. It reflects an ultimate form of tumor progression, probably associated with an accumulation of genetic changes. These will, however, still include the basic changes that have determined the specific development of the tumor. Assessment of the genetic constitution of sarcomatoid tumors may reveal genetic changes specifically assigned to one of the RCC subtypes, which, may lead to a correct diagnosis. In this paper we present the results of an analysis of a sarcomatoid RCC, by DNA flow cytometry, karyotyping, FISH and DNA studies, all performed on representative samples of the tumor tissue, in order to establish the specific subtype of this tumor and to shed light on the genetic events responsible for sarcomatoid transformation.

MATERIAL AND METHODS

Case history:

A 63-year-old male patient underwent radical nephrectomy for a RCC of the left kidney. A tumor measuring 6 x 4 cm was excised from the nephrectomy specimen. There was diffuse infiltration of the tumor into the adjacent kidney tissue and the tumor extended into the renal capsule. Lymph-node metastases were also observed. Microscopic examination revealed a high grade RCC with partly papillary and partly solid architecture. The cells were predominantly eosinophilic with atypical nuclei. Other areas consisted of spindle cells leading to a sarcomatous appearance. Figure 1a shows the highly differentiated chromophilic/papillary part of the tumor, whereas Figure 1b presents the part of the tumor with a histologically dedifferentiated appearance. A dedifferentiated chromophilic sarcomatoid RCC was diagnosed.

Methods:

Fresh representative tissue samples were submitted for cytogenetic investigation. Part of the tissue was cultured in RPMI 1640 supplemented with FCS (16%), glutamine and antibiotics for 5 to 7 days. The cultures were harvested and chromosome preparations were made according to standard cytogenetic techniques. The chromosomes were G-banded using pancreatin and karyotypes were described according to the ISCN'95 guidelines for cancer cytogenetics.

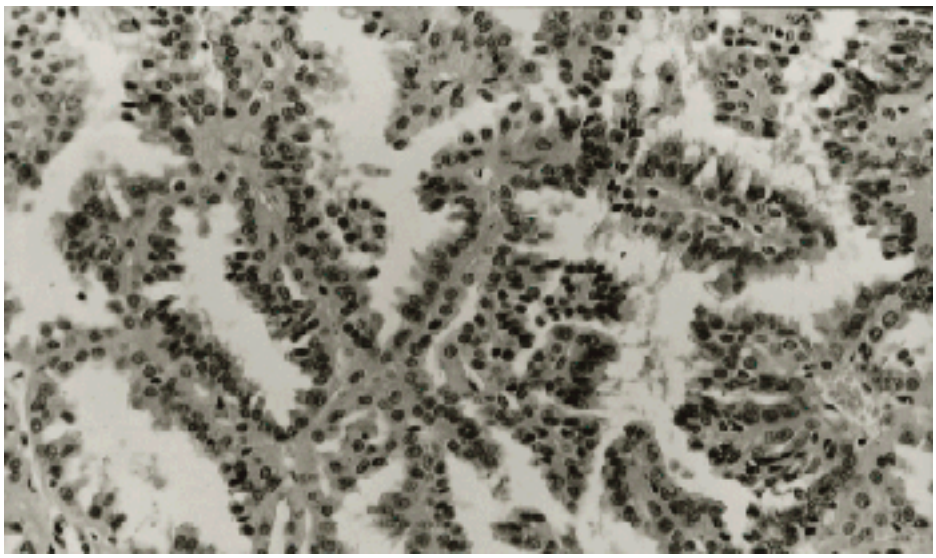

Figure 1a

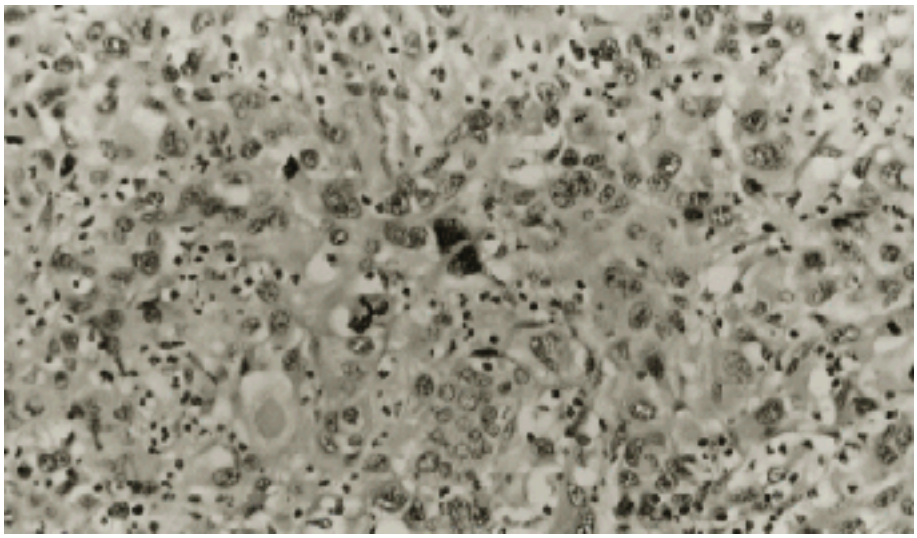

Figure 1b

Figure 1: Histology of the case showing the differentiated chromophilic papillary part of the tumor (204x) in a, and the dedifferentiated chromophilic solid part of the tumor in b

DNA flow cytometry was performed on single cell suspensions from frozen tissue, using trout red blood cells as an internal control [99]. For FISH studies, a chromosome 3-specific library (commercially available from ONCOR) was used. FISH was carried out according to the manufacturer's protocol.
Loss of heterozygosity (LOH) analysis of 3p was carried out as described [100]. *P53* mutation analysis was performed as described by Dijkhuizen et al [75]. Sequence analysis of PCR products was carried out as described by Van den Berg et al.[101].

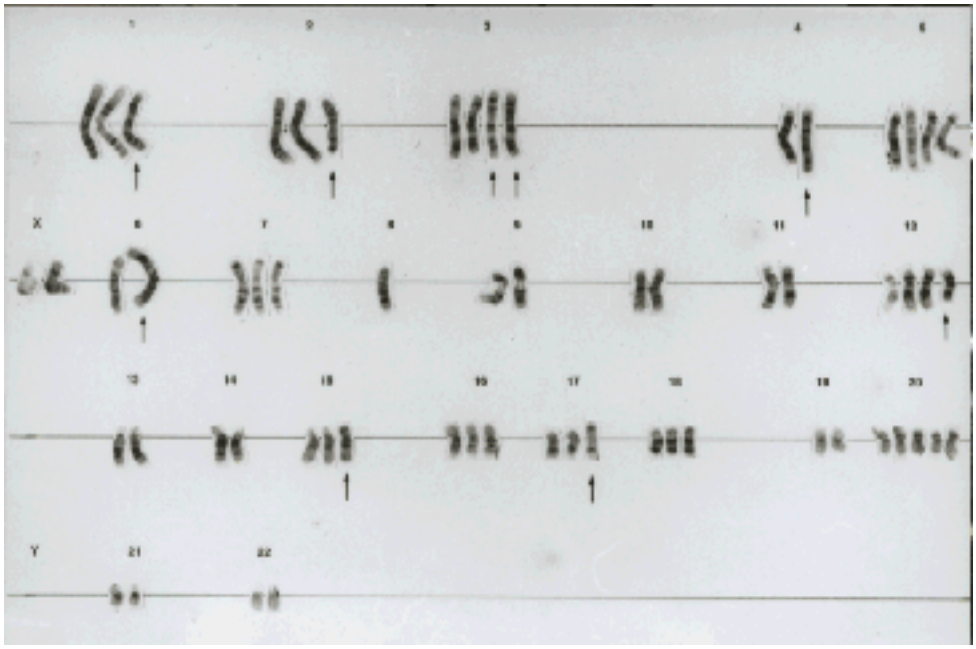

Figure 2: A karyotype of one of the metaphases of the present case revealing a 61,XX, -Y,add(1)(p32),der(2)t(2;18)(q14;q11),der(3)t(3;8)(q10;q10),+der(3)t(3;8)(q10;q10),-4, add(4)(q35),+5,-6,der(6)t(6;12)(p23;q13),-8,-8,-9,-10,-11,+der(12)t(6;12)(p12;q12),-13, -14,add(15)(p11),i(17)(q10),-19,+20,+20,-21,-22 chromosomal pattern.

RESULTS

Cytogenetics:

Cytogenetic analysis of 9 cells gave the following composite karyotype: 60-61,XX,-Y,add(1)(p32),der(2)t(2;18)(q14;q11),der(3;8)(q10;q10).ish der(3)(wcp3+), +der(3;8)(q10;q10).ish der (3)(wcp3+),-4,add(4)(q35),+5,-6,der(6)t(6;12)(p23;q13), -8,-9,-10,-11,+der(12)t(6;12)(p12;q12),-13,-14,add(15)(p11),i(17)(q10),-19,+20, +20,-21,-22[cp9]. Figure 2 shows a karyotype of one of the metaphases.

LOH analysis of 3p:

The markers D3S966, D3S1227, D3S1233, and D3S1101 were not informative in this case. For the informative markers D3S1038 at 3p25 and D3S1029 and *UBE1L* at 3p21 we detected LOH, whereas a retention of alleles was detected for D3S180 at 3p14. Most likely the 3p21-p25 region was lost in this tumor.

FISH analysis:

After FISH with a chromosome 3 library, we saw in metaphases with approximately 60 chromosomes, two normal appearing chromosomes 3 and one or two copies of a medio centric chromosome of which one arm and the centromeric region consisted of chromosome 3 material. None of the other chromosomes contained visible chromosome 3 material (Figure 3a). These results are in agreement with the cytogenetic findings. A second clone however, revealed the double amount of chromosomes, and besides the above mentioned normal and aberrant chromosomes 3, some smaller, unidentified chromosomes containing chromosome 3 material were observed (Figure 3b).

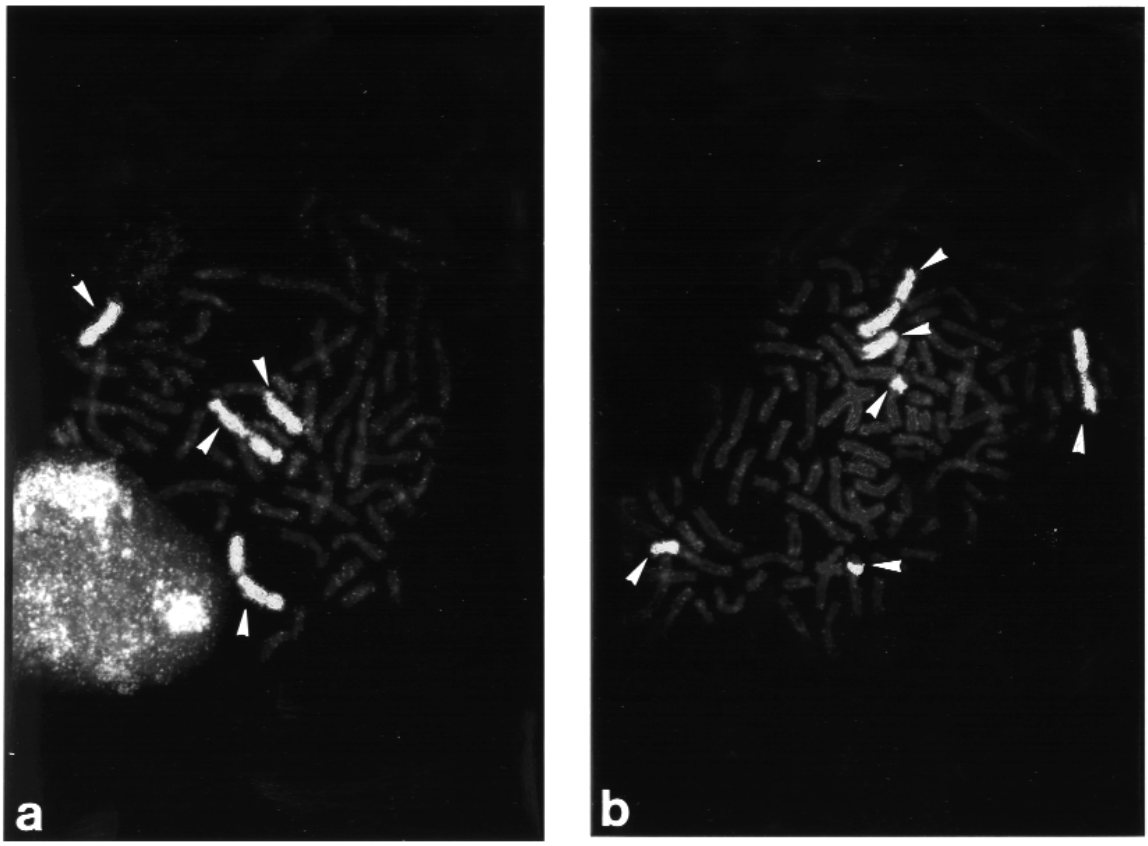

Figure 3: FISH results with a chromosome 3 library, showing the two different clones (in a and b respectively).

DNA-flow cytometry:

DNA-flow cytometry revealed a large diploid peak and a small peak corresponding with a near pentaploid chromosome number, most likley representing the distinct second clone discovered by FISH analysis. Flow cytometry failed to show the cytogenetically analyzed clone of approximately 60 chromosomes.

Mutation analysis of the p53 gene:

SSCP analysis of the whole coding region of the *p53* gene revealed an aberrant SSCP pattern upon analysis of exon 4 which was present only in the tumor and not in normal kidney tissue. Direct sequencing of this exon 4 PCR product indicated the presence of a T→G mis-sense mutation at base 856 (Figure 4). This resulted in a change of codon 113, TTC→TGC, leading to a Phe→Cys amino acid substitution.

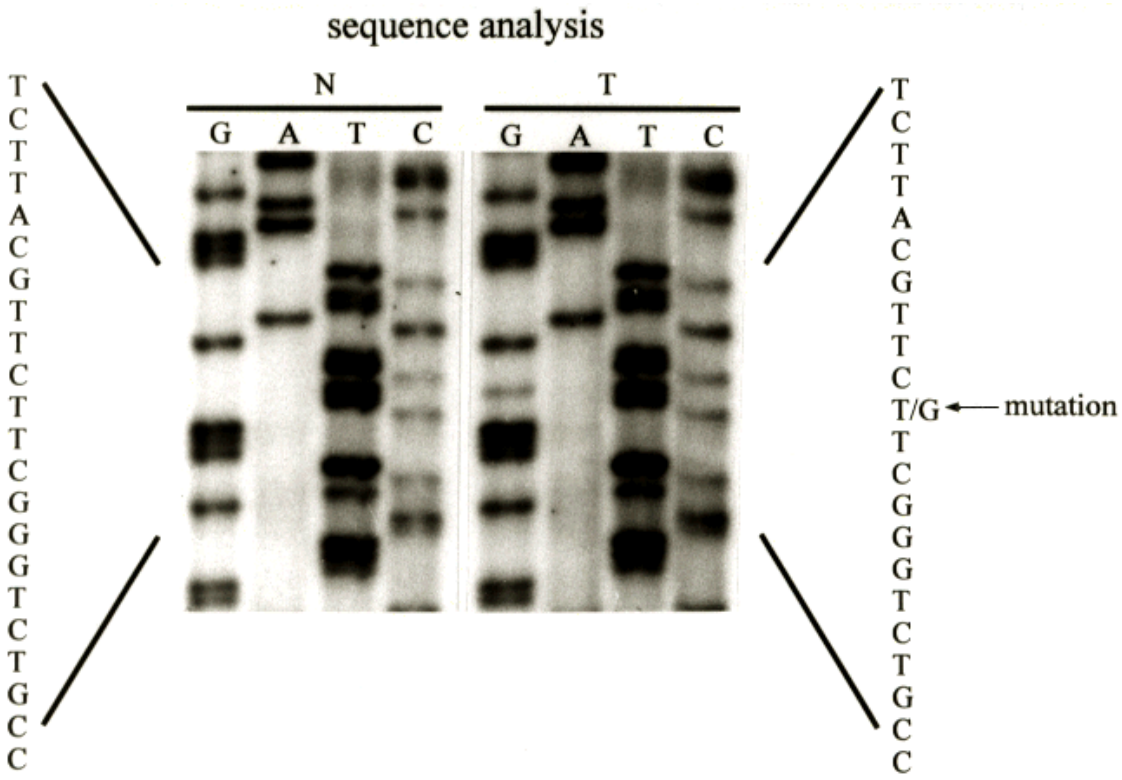

*Figure 4: Sequence analyses of exon 4 PCR products of the p53 gene. Direct sequence analyses of the exon 4 PCR products obtained upon amplification of DNA isolated from normal and tumor tissue. The arrowhead indicates the mutated base in the sequence gel. The arrow indicates the position of the T*fi *G mutation in the sequence. N, normal tissue; T, tumor tissue.*

DISCUSSION

Renal cell cancer comprises a heterogeneous group of tumors, the subtypes of which each have a distinct genetic constitution [24,26,28]. Sarcomatoid transformation can occur in most of the subtypes and represents a final stage of dedifferentiation. A diagnosis of these highly dedifferentiated entities, based on pathological criteria, may be difficult. Therefore genetic analysis of sarcomatoid RCC might be useful for a correct diagnosis of these neoplasms. Moreover, it might shed light on the genetic events responsible for this ultimate form of tumor progression and the subsequent aggressive behavior of these neoplasms. Limited information is available about the genetic constitution of sarcomatoid renal tumors. Genetic evidence has been found only for a clear cell origin. In two studies, nine out of twelve sarcomatoid tumors demonstrated the specific genetic change associated with the clear cell subtype, i.e. loss of (part of) 3p, confirming the histologic diagnosis of clear cell RCC [46,102]. The cytogenetic analysis of two cases of papillary sarcomatoid RCC has been described, but in these cases the histologic diagnosis was not confirmed by their genetic constitution [77,103]. The genetic events leading to sarcomatoid transformation in RCC are not known. Oda et al. [78] observed a relation between mutations of the *p53* gene and a sarcomatoid phenotype in eleven out of fourteen cases of sarcomatoid RCC. Of these, seven had a clear cell histology, five were mixed tumors, and two were diagnosed as granular. However, *p53* mutations have also been observed in high grade clear cell neoplasms without a sarcomatoid phenotype [85].

We performed a genetic characterization of a sarcomatoid RCC, diagnosed as chromophilic/papillary on the basis of the histology of the epithelial part of the tumor. We, therefore, expected the chromosomal pattern to show a combination of autosomal gains specific for the chromophilic or papillary subtype, i.e., +3q, +7, +12, +16, +17, and/or +20 [25,26,53,75]. Also loss of chromosome 17(p) was likely to be found since loss of 17(p) has been associated with progression to a higher grade in chromophilic carcinomas [75].

In the present case, no trisomy of chromosome 17 was observed. The karyotype did show an i(17)(q10), resulting in gain of the long arm and subsequent loss of the short arm of this chromosome, which could fit the diagnosis of a high grade chromophilic tumor. The presence of a mutation of the *p53* gene detected by SSCP analysis, however, was rather surprising, since mutations of this gene have been associated with progression and sarcomatoid transformation in clear cell neoplasms [78,85], but not in RCC of the chromophilic/papillary type [75].

Loss of 3p sequences is specific for clear cell RCC and chromophilic or papillary tumors do not usually show this aberration [23,26,28]. In the present case, two copies of a der(3)t(3;8)(q10;q10) were found resulting in loss of the short arm of chromosome 3. For 3p21-p25, loss of 3p sequences was confirmed by molecular analysis. FISH analysis excluded the presence of chromosome 3 material in other rearrangements, but also led to the detection of a second clone with a chromosome number in the pentaploid range. In this clone, in addition to the der(3) mentioned above, rearrangements containing chromosome 3 material were seen, explaining the differences between cytogenetic and molecular analysis concerning 3p losses. Reexamination of the chromosome slides revealed a few cells with a pentaploid chromosome number. The quality of these metaphases, however, did not allow a karyotypic analysis. Probably, there has been in vitro overgrowth by the near-triploid cells, which were present in the original tumor, but in a number too small to be detectable by DNA flow cytometry. The fact remains that both clones showed loss of

3p sequences, which is thought to be specific for the clear cell type. The above mentioned findings are highly suggestive for a clear cell origin of the present sarcomatoid tumor, and indicate the limitations of a purely histological diagnosis, according to which the tumor was defined as chromophilic.

Tumor progression results from an accumulation of genetic changes. Although the development of chromophilic or papillary RCC is associated with alterations distinct from those associated with the development of clear cell tumors, a substantial number of secondary changes, mostly related to tumorprogression, are similar in both subtypes [26,28,46,53,104-108]. Common changes are loss of 6q, 9, 11, 14q, and 17p, and gain of chromosomes 12 and 20. In addition, clear cell RCC specifically shows gain of 5q, loss of 8p, 10q, 13, and 18q, and mutations of the *p53* gene, whereas the chromophilic or papillary subtype shows gain of 3q, 8, and 16, and loss of 21. Clonal changes found in the present case include gain of chromosomes 5, 12, and 20, loss of 6q, 8p, 9, 10, 11, 13, 14, and 17p and mutations of the *p53* gene. These comprise changes common to both subtypes as well as changes specific for clear cell RCC, again pointing to a clear cell origin.

Whether a certain total amount of genetic events or the formation of specific changes is necessary for creating a sarcomatoid phenotype is not yet known. A few comments, in relation to the present findings, may be made. Mutations of the *p53* gene are more generally found in high grade clear cell tumors [85], and a relation has been suggested between *p53* mutations and sarcomatoid transformation of clear cell RCC [78]. Since *p53* mutations also occur in high grade clear cell tumors without sarcomatoid components, this genetic change in itself will not create a sarcomatoid phenotype. Loss of chromosome 14 has been associated with tumor progression in clear cell as well as in chromophilic or papillary tumors. In a recent paper Thrash-Bingham et al. [104] found a relation between loss of 14q and increased genetic instability, thus facilitating the formation of additional chromosome rearrangements, leading to a more malignant phenotype. The observed rearrangements of chromosome 1, the der(2)t(2;18), and the loss of chromosome 4 sequences have not been specifically assigned to any of the RCC subtypes and might, therefore, be important in the progression to a sarcomatoid tumor. Due to the complexity of the chromosomal pattern in our case, however, it remains difficult to distinguish alterations which are directly related to sarcomatoid transformation.

Taken together, the genetic rearrangements observed in the present case, especially loss of 3p sequences and *p53* mutations, point to a clear cell rather than to a chromophilic origin. A clear cut answer about the origin of this tumor has to await identification of the distinct genes responsible for the development of the different RCC subtypes, but conceivably, the present case started out as a mixed clear cell/chromophilic tumor in which the clear cell part progressed to sarcomatoid RCC, whereas the chromophilic tumor cells remained carcinomatous. This explains the chromophilic appearance of the carcinomatous area of the tumor. Our findings indicate that the histology of the carcinomatous component not necessarily reflects the true nature of a sarcomatoid RCC. The genetic consititution may be a more reliable guide to a correct diagnosis.

CHAPTER 3.2

3.2 CHROMOPHILIC RENAL CELL CANCER, WITH TRANSLOCATIONS INVOLVING BREAKPOINT Xp11.2

CHAPTER 3.2.1

DISTINCT Xp11.2 BREAKPOINTS IN TWO RENAL CELL CARCINOMAS EXHIBITING X;AUTOSOME TRANSLOCATIONS

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ABSTRACT

Several human renal cell carcinomas with X;autosome translocations have been reported in recent years. The t(X;1)(p11.2;q21) appears to be a specific primary anomaly, suggesting that tumors with this translocation form a distinct subgroup of RCC. Here we report two new cases, one with a t(X;10)(p11.2;q23), the other with a t(X;1)(p11.2;p34). The common breakpoint in Xp11.2 suggests that they belong to the above-mentioned subset of RCC. Using FISH in conjunction with X-specific YAC clones, we demonstrate that the two new cases exhibited distinct breakpoints within Xp11.2.

INTRODUCTION

Renal cell carcinomas (RCCs) arise from tubular epithelium. The incidence of RCC peaks in the sixth decade of life[109]. Approximately 70% of the patients are males. This disparity between the sexes is even more striking when the tumor occurs at an early age.

Cytogenetic and molecular genetic investigation of RCC have revealed consistent karyotypic abnormalities corresponding to the different histologic RCC subtypes [23,25,53]. A deletion of the short arm of chromosome 3 occurrs in the clear cell type which is the most common type of RCC. Also a (partial) trisomy of chromosome 5, especially the

5q22-qter segment, is frequently found in the clear cell tumors. A different set of abnormalities is found in the chromophilic (or granular), tubulopapillary tumors. Instead of 3p and 5q aberrations, this subtype is characterized by combinations of gain of chromosomes 7 and 17 and loss of the Y chromosome, together with additional copies of chromosomes 12, 16 and/or 20. Renal oncocytoma shows loss of the Y chromosome in combination with loss of chromosome 1. Recently a subset of oncocytomas was described with abnormalities involving 11q13 [69]. In the chromophobic tumor type loss of the Y chromosome and loss of chromosomes 1, 6, 14, 15, and 22 may be found. In addition, telomeric associations are observed in this subtype.

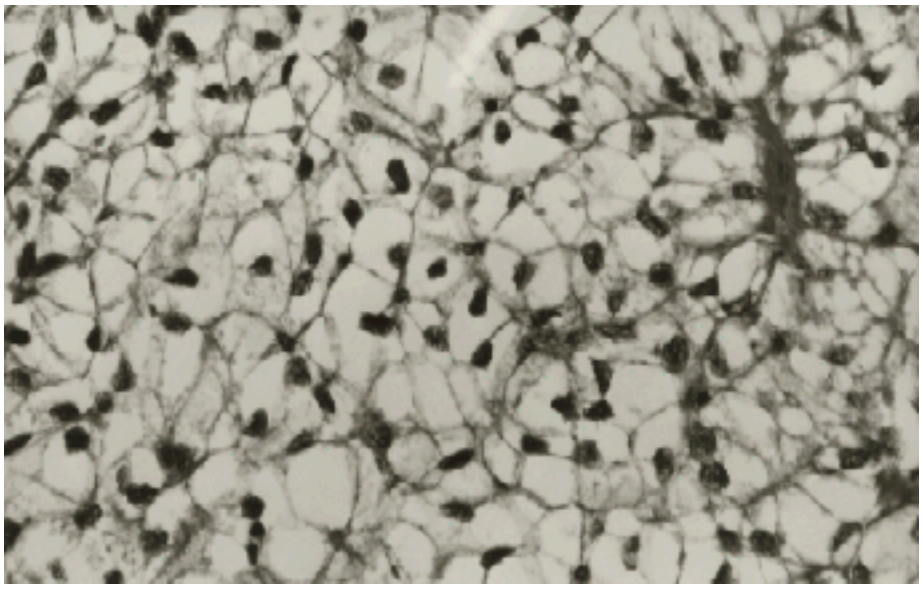

Figure 1a

Figure 1b

Figure 1: Histology of case 1 showing the clear cell type of this tumor with areas of a) a compact (solid) growth pattern, and b) a papillary growth pattern (HE, 480x).

Meloni et al. [58] described four cases of papillary RCC in which a t(X;1)(p11.2;q21) was observed, in one case as the sole abnormality. This unique translocation had first been decribed by de Jong and co-workers in 1986 in a case of RCC in a 2-year-old boy [9]. In addition, three other cases of RCC have been published [6,8,110], two containing
X;autosome translocations [6,110] and one with a del(X) [8], again with breaks in Xp11.2. This suggests that tumors carrying Xp11.2 anomalies represent a new subgroup of RCC.
We present two new cases of RCC carrying translocations involving Xp11.2. Using FISH in conjunction with Xp11.2-specific YAC clones, we determined the relative locations of the Xp11.2 breakpoints in these tumors. The results obtained were compared with data from the literature, and attempts were made to correlate the different breakpoints with tumor histology.

MATERIAL AND METHODS

Patient Material and Cytogenetic analysis:

The first patient was a 52-year-old male, who presented with a 12 cm in diameter RCC in the left kidney. The tumor had a papillary growth pattern (Fig 1b), but also showed distinct clear cell features (Fig 1a). The second patient was a 77-year-old male, with a 3x2 cm RCC in the left kidney. This tumor was of the chromophilic papillary type, grade II. Both tumors were classified according to Thoenes et al.[20].

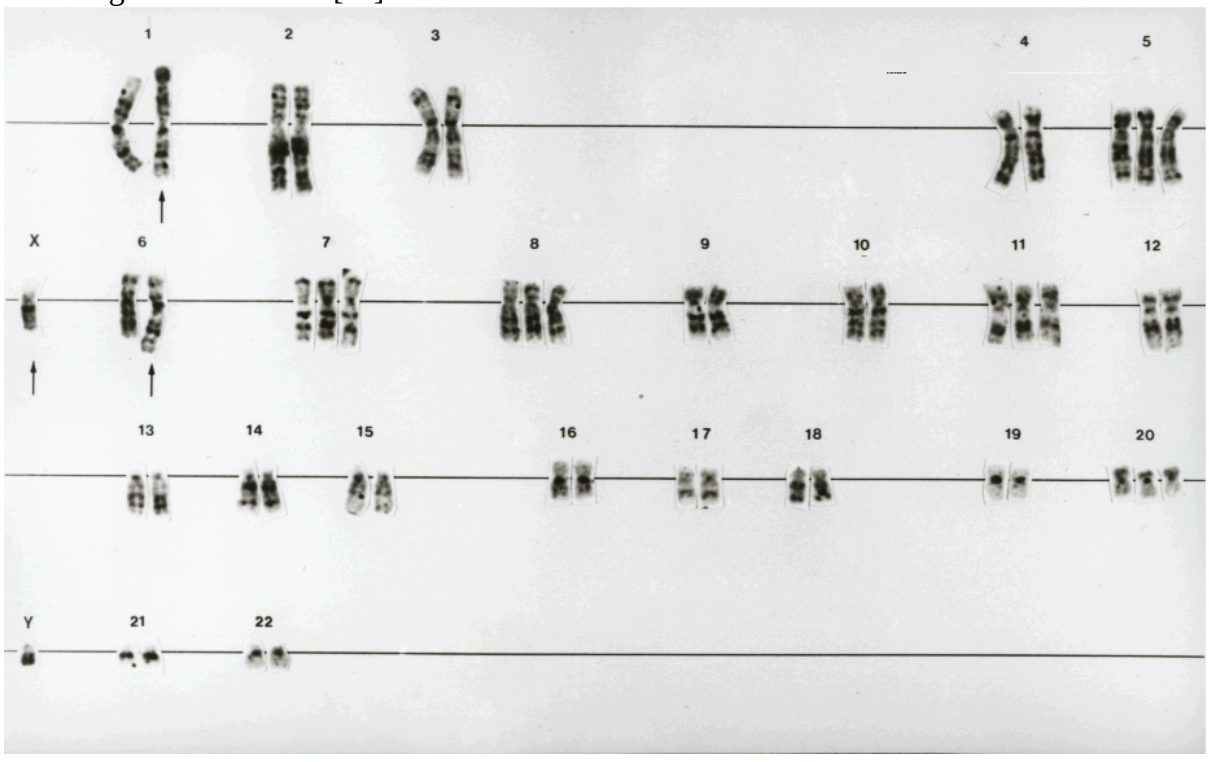

Figure 2a: One of the metaphases of case 1 revealing the 51,Y,t(X;1)(p11.2;p34),+5,der(6) t(1;6)(q11;q11),+7,+8,+11,+20 chromosomal pattern.

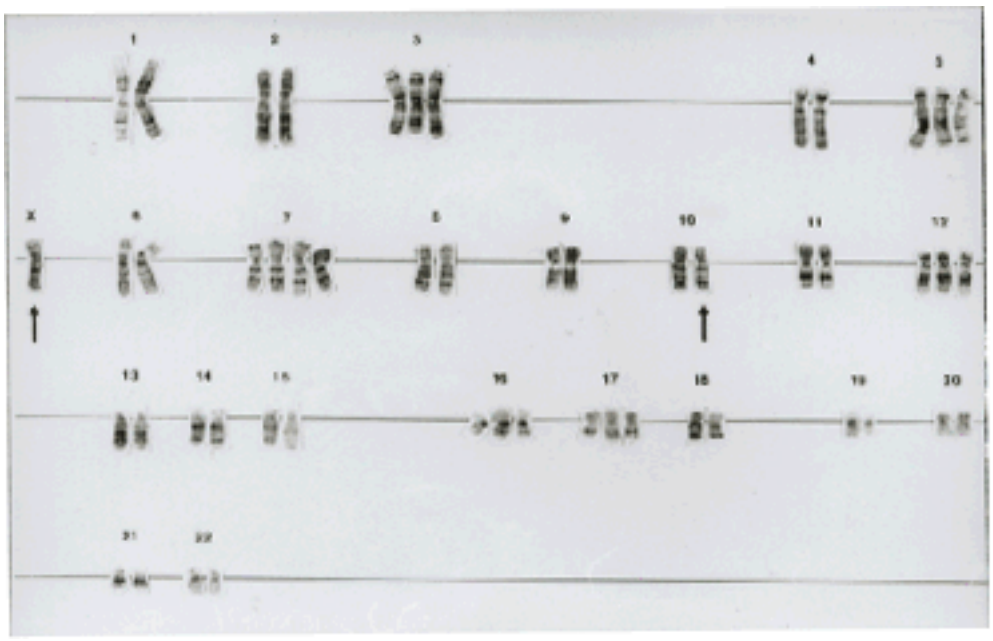

Figure 2b: One of the metaphases of case 2 revealing the 52,-Y,t(X;10)(p11.2;q23), +3, +5,+7,+7,+12,+16,+17 chromosomal pattern.

Fresh representative samples of both tumors were submitted for cytogenetic investigation. Part of the tissue was cultered for 5-7 days in RPMI 1640 supplemented with FCS (16%), glutamine and antibiotics. The cultures were harvested and chromosome preparations were made according to standard cytogenetic techniques. The chromosomes were G-banded using trypsin and karyotypes were described according to the ISCN 1991.

Fluorescence in situ Hybridization:

All fluorescence in situ hybridization (FISH) procedures on metaphase spreads were essentially as described previously [111]. The probes used were: the centromere X-specific alphoid sequence probe pBamX5, the OATL1 YAC #2 and the OATL2 YAC #7. All probes were labeled with either biotin-11-dUTP (Sigma, St. Louis, MO) or digoxigenin-11dUTP (Boehringer Mannheim, Germany) using a nick-translation kit (GIBCO Life Technologies, Gaithersburg, MD). The labeled DNA was precipitated in the presence of sonificated herring sperm DNA (50-fold concentration). In the case of the YACs, a 50-fold amount of sonicated total human DNA was coprecipitated for preannealing purposes. This mixture was dissolved in 6 µl of a hybridization FDST solution (50% v/v deionized formamide, 10% w/v dextran sulphate, 2 x SSC, 1% Tween-20, pH 7.0). Prior to hybridization, the probe was denatured at 80°C for 10 min, chilled on ice, and incubated at 37°C for 4 hrs (200 ng YAC DNA per reaction) allowing preannealing. In case of centromeric probes, no preannealing was performed and the probe concentration was 10 ng per hybridization. Metaphase spreads were prepared using standard procedures. The slides were pretreated with RNase A (100 µg/ml in 2 x SSC at 37°C for 1 hr). Subsequently, the slides were

denatured in 70% formamide, 2 x SSC, pH 7.0 at 70°C for 2 min and incubated with the probes under an 18x18-mm coverslip in a moist chamber for 2 days.
Immunocytochemical detection of the hybridizing probes was achieved using FITC-conjugated avidin (Vector laboratories, Burlingam, CA; 1:500 diluted) followed by a two time amplification using rabbit anti-FITC (Vector laboratories; 1:250 diluted), and mouse anti-rabbit conjugated FITC in case of biotinylated probes (green signals). For digoxigenin-probes, rhodamin-conjugated sheep anti-digoxigenin (Boehringer Mannheim: dilution 1:20) was used followed by Texas Red-conjugated donkey anti-sheep (red signals) (Jackson Immunoresearch, West Grove, PA; dilution 1:100). The slides were mounted in antifade medium (9 parts 2.3% w/v di-azobicyclo-(2,2,2)-octane, DABCO; Merck, Darmstadt,

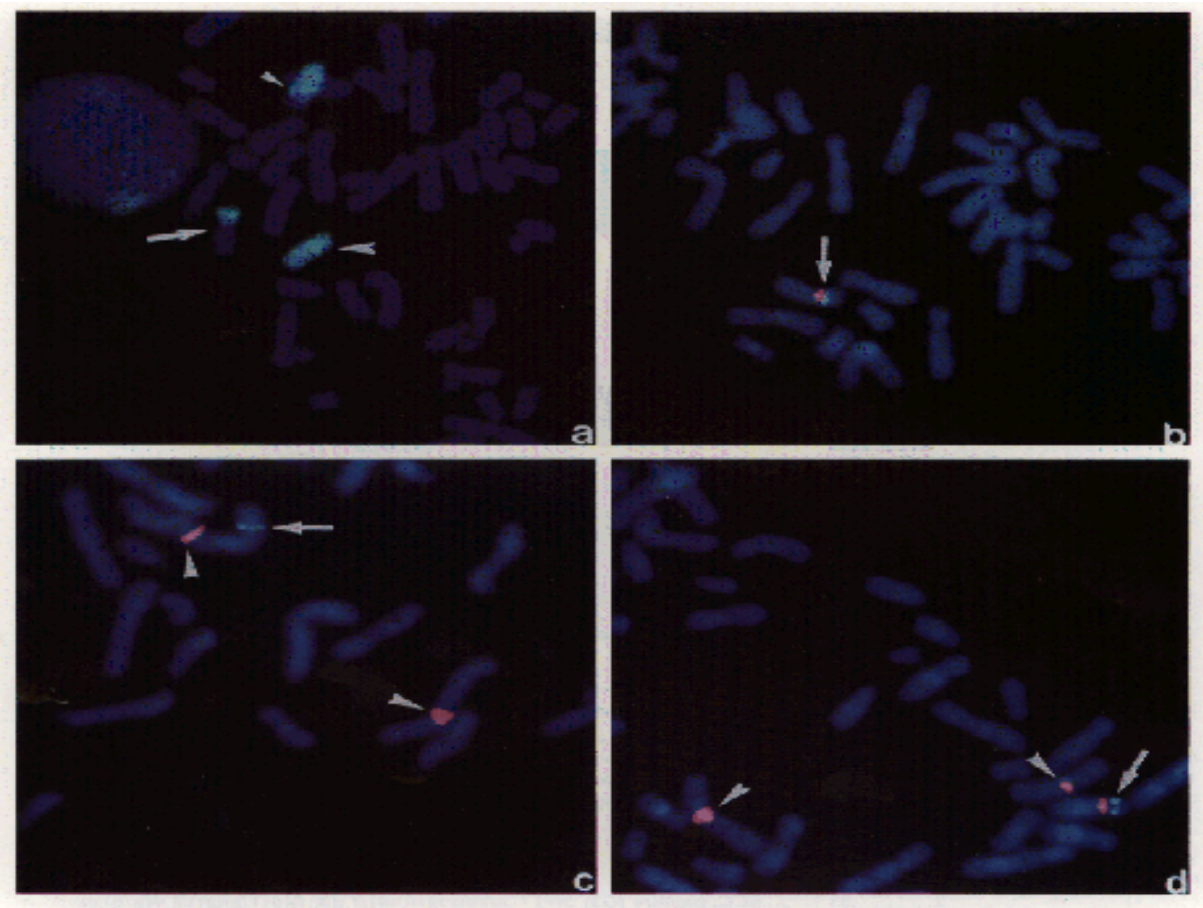

Figure 3: FISH on case 2 (a,b)- and case 1 (c,d)-derived metaphase spreads using a paint for chromosome 10 (a; green), OATL1 YAC2 (b,c;green) or OATL2 YAC7 (d; green), in conjunction with the chromosome X centromere probe (b; red), the chromosome 1 centromere probe (c; red), or a combination of chromosome 1 and X centromere probes (d; red). In a: the normal 10, the der(10) and the der (X) are marked by a large arrowhead, a small arrowhead, and an arrow, respectively. In b: the der (X) is marked by an arrow. In c and d: the chromosome 1 centromeres are marked by arrowheads, whereas the YAC signals are indicated by arrows. In b and d: the X centromeres (red) are not marked. Chromosomes are counterstained in blue (DAPI).

Germany in glycerol and 1 part 0.2 M TRIS/HCl, pH=8.0) containing 4,6-diamino-2-phenylindole (DAPI, 0.5 µg/ml, Sigma) for counterstaining of the chromosomes.
Slides were viewed under a Zeiss Axiophot epifluorescence microscope equipped with appropriate filters for the visualization of FITC, Rhodamin/Texas Red, and DAPI fluorescence as well as the simultaneous visualization of FITC and Texas Red fluorescence (Omega double band pass filter, Brattlesboro, VT), Separate digital images (for Texas Red, FITC and DAPI, respectively) were recorded using a Photometrics high-performance CH250/A cooled CCD-camera interfaced onto a Macintosh IIci computer. The images were superimposed and displayed in red-green-blue pseudocolors on the computer screen using the image analysis and processing software program BDS-image (Biological Detection Systems, Rockville, MD). Photographs were made from the computer screen on Kodak EPP 100 plus colorslide film using a Polaroid Quickprint.

RESULTS

Banding Analysis:

A total number of 10 cells were analyzed from case 1. Eight cells showed a balanced translocation involving chromosomes X and 1, as well as other changes, giving the karyotype 51,Y,t(X;1)(p11.2;p34),+5,der(6)t(1;6)(q11;q11),+7,+8,+11,+20 (Fig. 2a).
Eleven cells were analyzed from case 2. The karyotype was 52,-Y,t(X;10)(p11.2;q23), +3,+5,+7,+7,+12,+16,+17 (Fig. 2b). The constitutional karyotype of both patients was 46,XY.

FISH Analysis:

Two OAT-specific YACs, one corresponding to the OATL1 (YAC #2; insert size 500 kb)- and the other corresponding to the OATL2 (YAC #7; insert size 600 kb)-cluster on the human X chromosome, were used. Metaphase spreads of both tumors were analyzed with these YACs in conjunction with FISH. The results of the FISH analyses are given in Figure 3. In case 1, all sequences contained within YAC #2 were translocated to chromosome 1 in the X;1-translocation (Fig. 3c), whereas all sequences contained within YAC #7 were retained on the X chromosome (Fig. 3d). The breakpoint of case 1 is, therefore, distal to the OATL2 locus and proximal to the OATL1 locus (Fig. 4). For case 2, all sequences contained within YAC #2 and YAC #7 were retained on the X chromosome (Fig. 3a and 3b), indicating that the X;10-translocation in this case was distal to the OATL1 locus (Fig. 4).

DISCUSSION

The cytogenetic and histologic data of all cases of RCC with X;autosome translocations are summarized in Table 1. Histologically most of the cases were described according to the WHO-classification. They include four papillary tumors [58], one clear cell tumor [6] and one metastasis of a papillary tumor [8]. Three of the cases were classified according to the criteria proposed by Thoenes and Störkel ([9], the present two cases). Two of them revealed a papillary growth pattern with distinct clear cell features, whereas the third tumor (case 2) was a chromophilic tubulopapillary RCC.

Table 1:Histologic and Cytogenetic data of RCC with X;autosomal translocations

REF.	CASE	AGE (y)	HISTOLOGY	KARYOTYPE
[9]		2	Clear cell; papillary+com-pact	46,Y,t(X;1)(p11.2;q21)
[6]		1	Clear cell; Alveolar	46,Y,t(X;17)(p11.2;q25)
[8]		24*	RCC; Papillary	45,Y,del(X)(p11),del(11)(q23?),add(13)(p11),+add(13)(p11),add(16)(p11),-17,-18
[110]		-	-	46,Y,t(X;1)(p11.2;p34.1)
[58]	1	68	RCC; Granular + Papillary (G4)	49,Y,t(X;1)(p11.2;q21),+7,+15,+17
	2	55	RCC; Papillary (G3)	41,Y,t(X;1)(p11.2;q21),i(1)(q10),der(3)t(3;13)(p12;q12),-4,-5,inv(7)(q11.2 p22),-9,-10,-11,-13,add(16),+17,+18,+20/40,idem,-Y
	3	-	RCC; Papillary (G3)	45,t(X;1)(p11.2;q21),-22
	4	24	RCC; Papillary	46,Y,t(X;1)(p11.2;q21)/46,idem,inv(13)(q12q22)
This series	1		Clear; Papillary + compact (G2)	51,Y,t(X;1)(p11.2;p34),+5,der(6)t(1;6)(q11;q11),+7,+8,+11,+20
	2		Chromophilic; Papillary (G2)	52,-Y,t(X;10)(p11.2;q23),+3,+5,+7,+7,+12,+16,+17

*Metastasis

The remaining case was published by Yoshida et al. [110] but lacks a description of the pathologic findings. Cytogenetic analysis revealed a t(X;1)(p11.2;q21) in five out of ten cases [9,58], in two of them as the sole anomaly. This led to the conclusion that these tumors might represent a new cytogenetic subtype of RCC, especially since this abnormality has never been described in any other malignancy. The other cases exhibited a t(X;1)(p11.2;p34) ([110], our case 1), a t(X;17)-(p11.2;q25) [6], a t(X;10)(p11.2;q23) (our case 2) and a del(X)(p11) [8].

The common denominator in cases of RCC with X;autosome translocations is the breakpoint in Xp11.2, suggesting an important role for this region in tumor development. Cytogenetically, a similar breakpoint in Xp11.2 has been observed in synovial sarcoma, with the specific translocation t(X;18)(p11.2;q11.2). Recently, de Leeuw and Suijkerbuijk [111-114] mapped two alternative Xp11.2 breakpoints in these neoplasms to the OATL1 and the OATL2 regions, respectively, using

FISH and Xp11.2-specific YAC clones. The genes at the OATL1/OATL2 regions as well as that at 18q11.2 have recently been cloned [115,116].

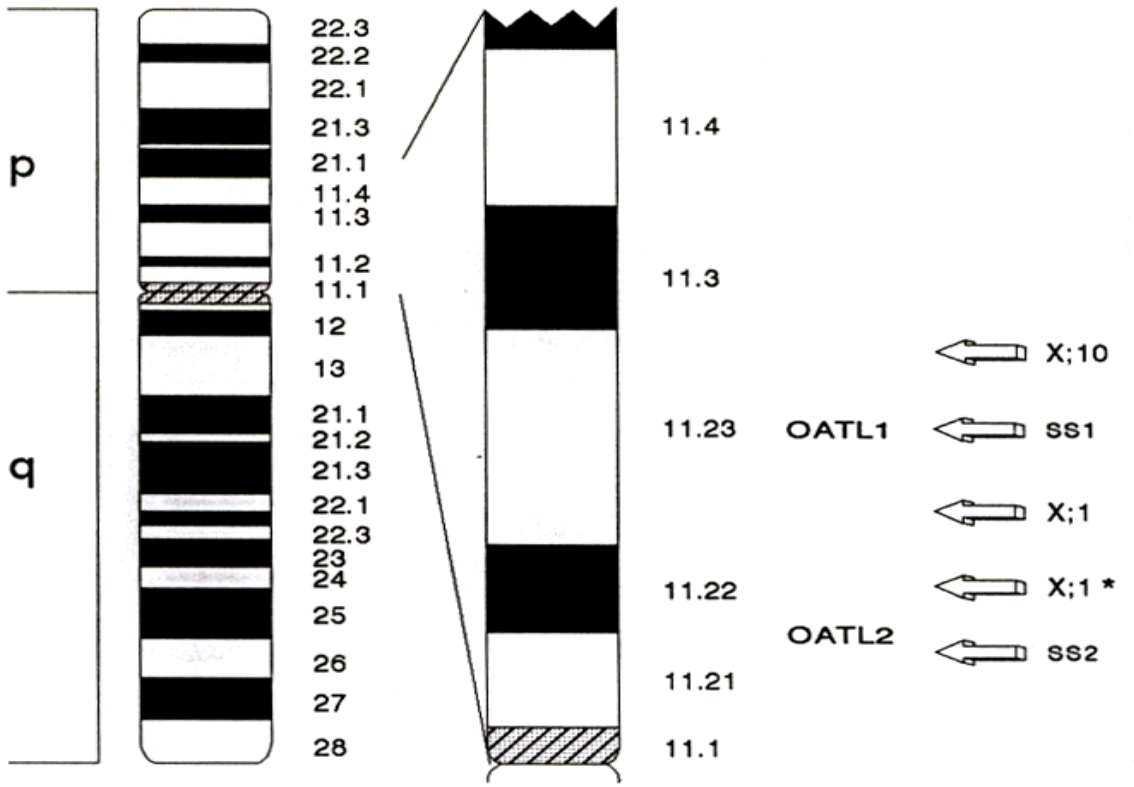

Figure 4: Diagram representing the short arm of the X chromosome and the relative locations of the different breakpoints in Xp11.2 (arrows); SS1 and SS2, two alternative synovial sarcoma-associated breakpoints; X;1, t(X;1)(p11.2;q21) breakpoint observed in papillary renal cell carcinoma ; X;1 and X;10, two breakpoints corresponding to the new cases reported here, respectively.*

In order to determine the relative breakpoint positions at Xp11.2 in the RCCs, four cases ([112], and the present two cases), were examined with the OATL1 and OATL2-specific YACs and other probes [112,117]. The examination of the first two cases revealed a breakpoint location distal to the OATL2 and proximal to the OATL1 region [112]. Surprisingly, the relative breakpoint of at least one of the present two cases appears to be different. In case 2, it is located distal to both the OATL1 and OATL2. In case 1 the relative breakpoint is distal to the OATL2 and proximal to the OATL1 region. The YAC#7 signal, which was previously found to be split by the translocation in RCCs with a t(X;1)(p11.2;q21) [112], is retained on the X chromosome in case 1.
Since Xp11.2 contains various cross-hybridizing low copy repeated motifs [117], the breakpoint in case 1 may be similar to the one found by Suijkerbuijk et al. [112]. This suggests that there is a

subtype of RCC with this specific Xp11.2 breakpoint, possibly with a distinct histology, involving both clear cell features and a papillary growth pattern.
Case 2 was a chromophilic tubulopapillary RCC without any clear cell features, and had, besides the Xp11.2 translocation, a chromosomal pattern frequently associated with this subtype [23,53], i.e. -Y,+7,+7,+12,+16, and +17. Moreover, the FISH results indicate that the breakpoint in this case was located distal to the OATL1 region, whereas the breakpoints in the other three cases examined with FISH are located between the OATL1 and OATL2 regions. In this case the Xp11.2 translocation might be coincidental.
Alternatively, distinct sequences may be involved in the development of specific subsets of renal tumors, analogous to the findings in synovial sarcoma. In these sarcomas, two related regions in Xp11.2 are involved in the translocation with chromosome 18. This alternative involvement seems to correlate with two histologic subtypes of this neoplasm. Biphasic synovial sarcomas display a break predominantly in the OATL1 region whereas the monophasic subtype appears to be associated with the OATL2 region [111,116]. It is tempting to speculate that in RCC the breakpoint differences may also reflect subtle differences in tumor histology. The other cases were classified according to the criteria of the WHO [118], and the cell type is not mentioned. Reexamination of these cases, using the classification of Thoenes and Störkel [20], might reveal similar histologic patterns to our case 1.
An interesting finding is that four of ten patients with an RCC carrying an X;autosome translocation were of relative young age (1, 2, 24, and 24 years old). Overall, the incidence of RCC increases with age. In all of these young patients, except one, the X;autosome translocation was the sole aberration, indicating its crucial role in tumorigenesis. The fourth case, published by Ohjimi et al. [8], had a del(X) and was a metastasis. The additional chromosomal abnormalities found by the authors are probably a reflection of the metastatic potential of this tumor. The case described by Yoshida et al. [110] also had t(X;1) as the sole aberration, but the age of the patient was not provided.
Thus far, all RCC with Xp11.2 abnormalities have developed in male patients. Whether or not a X linked tumor suppressor gene is involved, as proposed by Tomlinson et al. [6] and Meloni et al. [58], is not clear. The fact that all of the cases developed in males should perhaps not be overrated since 70% of all cases of RCC occur in males and, including the present cases, only 10 cases have yet been published. RCC in male patients frequently show loss of the Y chromosome. This is especially true for the chromophilic (papillary) type, in which Y chromosome loss is found in 84% of cases [82]. In the cases of RCC with Xp11.2 abnormalities, Y chromosome loss is found in only 20% of the cases. This percentage closely resembles the findings in clear cell RCC, in which the Y chromosome is lost in 22% of male patients. There might be a relation between this observation and the finding of at least three tumors with distinct clear cell features.
In conclusion: RCCs with a breakpoint Xp11.2 may represent a new subtype of RCC with a distinct histology and an aberrant age distribution. A more detailed description of the pathologic findings of these neoplasms, in addition to the exact determination of the breakpoints and genes involved in Xp11.2, might shed light on this subject.

CHAPTER 3.2.2

DISTINCT FEATURES FOR CHROMOPHILIC RENAL CELL CANCER WITH Xp11.2 BREAKPOINTS

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Cancer Genetics and Cytogenetics: submitted

ABSTRACT

Chromophilic renal cell cancer, revealing translocations involving chromosome X, breakpoint Xp11.2 has been recognized as a variant of chromophilic/papillary RCC. For several years it has been thought that these tumors develop exclusively in males, but recently two female cases have been described. A literature review disclosed one other female case, in which only the der(1)t(X;1) was present. In this paper, we describe the fourth female case of a chromophilic/papillary RCC showing a t(X;1)(p11.2;q21). Including the present case, a total of 15 tumors have been described, leading to a moderate male preponderance, which is quite different from the high male preponderance observed in ordinary chromophilic/papillary renal cancer. In addition, these neoplasms have a distinct morphological appearance, and show a tendency to develop in children and young adults. These findings emphasize that chromophilic renal tumors, exhibiting translocations involving breakpoint Xp11.2, are a distinct subtype of renal cancer.

Chromophilic or papillary renal cell cancer (RCC) is a subtype of RCC, comprising 10%-15% of cases. These tumors find their origin in the proximal part of the tubules, and usually have a papillary growth pattern. Chromophilic/papillary RCC shows a male preponderance, the male to female ratio is approx. 8:1. Genetic data reveal that the majority of cases have a specific combination of autosomal trisomies, of which +7 and +17 are the most consistent findings [26,75].
A small subset of chromophilic/papillary RCC is characterized by translocations involving Xp11.2, in some cases accompanied by other changes, including trisomy 17[8,56,58,59,119,120]. In others it is the sole cytogenetic change [6,7,9,109,121]. A literature review is summarized in Table I.

Table I: Cytogenetic data of Xp11.2 positive chromophilic RCC

Reference	Age	Sex	Cytogenetics
[6]	1.5	m	46,Y,t(X;17)(p11.2;q25)
[7]	8	m	46,Y,t(X;17)(p11.2;q25)
[109]	-	m	46,Y,t(X;1)(p11.2;p34)
[56]	52	m	51,Y,t(X;1)(p11.2;p34),+5,der(6)t(1;6)(q11;q11),+7,+8,+11,+20
[8]	24	m	45,Y,del(X)(p11),del(11)(q23?),add(13)(p11),+add(13)(p11),add(16)(p11),-17,-18
[59]	-	f	64~81,t(X;1)(p11.2;q21) and other changes
[119]	-	f	45,X,-X,der(1)t(X;1)(p11.2;p34.3),der(16)t(1;16)(p34.3;q24),der(19)t(X;19)(q13;p13.1)
[58]	68	m	49,Y,t(X;1)(p11.2;q21),+7,+15,+17
	55	m	41,Y,t(X;1)(p11.2;q21),i(1)(q10),der(3)t(3;3)(p12;q13),-4,-5,inv(7)(q11.1;p22),-9,-10,-13,add(16),+17,-18,+20/40,idem,-Y
	-	m	45,Y,t(X;1)(p11.2;q21),-22
	24	m	46,Y,t(X;1)(p11.2;q21)/46,idem,inv(13)(q12q22)
[120]	15	m	49,Y,t(X;1)(p11.2;q21),+der(X)t(X;1)(p11.2;q21),+5,-16,+17,+18
[9]	2	m	46,Y,t(X;1)(p11.2;q21)
[121]	29	f	46,X,t(X;1)(p11.2;q21)
our case	69	f	44~45,X,t(X;1)(p11.2;q21),-5,add(7)(p22),r(8)(p23;q43),der(14)t(5;14)(q11;p12)

A specific t(X;1)(p11.2;q21) has been observed in eight cases thusfar, and recently the genes involved in this translocation have been cloned [121,122]. The translocation results in a fusion of the transcription factor TFE3 on the X-chromosome, to a novel gene, designated PRCC on chromosome 1. The reciprocal fusion products are both expressed in these neoplasms [121]. Variants of the t(X;1)(p11.2;q21), in which Xp11.2 is translocated to 1p34 or 17q25 have been

described in two cases each. The genes involved in these variant translocations have not been identified yet, but most likely they share sequence homology with the PRCC gene at 1q21, resulting in fusion genes with a similar function as the one in the t(X;1)(p11.2;q21) [123].

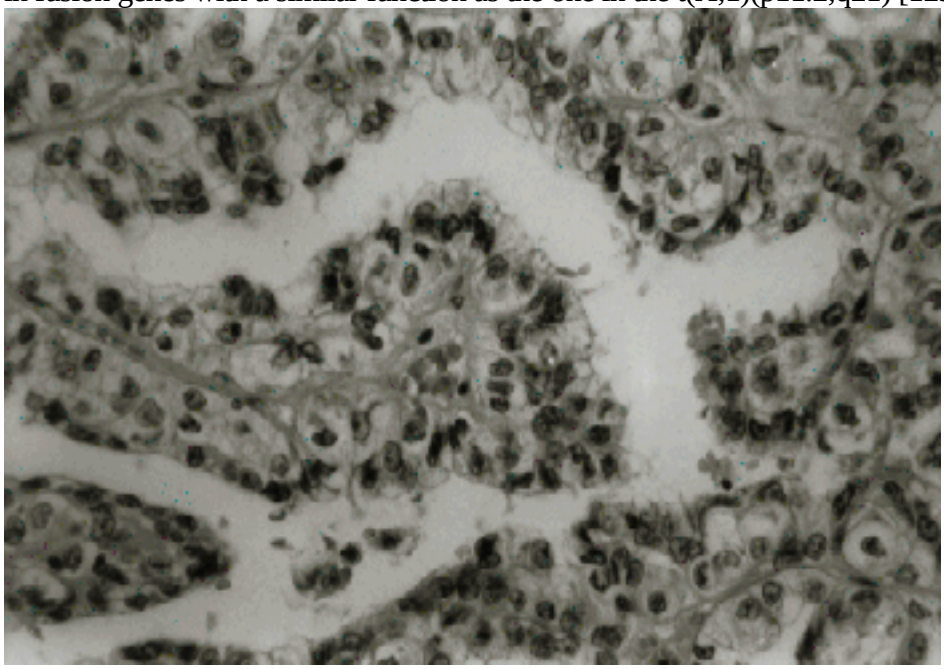

Figure 1: Histology of the present tumor

For several years the Xp11.2 chromophilic or papillary tumors have been thought to arise exclusively in males. Recently, however, two female t(X;1)-positive tumors have been published [59,121]. A third case was published by Kovacs et al. [119], but in this case only the der(1)t(X;1) was present, aside from one normal appearing chromosome X. We present the fourth case of a chromophilic/papillary RCC, in a female patient, showing a t(X;1)(p11.2;q21). The patient was a 69 year old female. The tumor tissue was composed of tumor cells with a clear cell appearance, arranged in a papillary architecture (Figure 1). Cytogenetic analysis of eleven cells of the tumor tissue revealed a 44~45,X,t(X;1)(p11.2;q21),-5,add(7)(p22),r(8)(p23;q24),der(14)t(5;14)(q11;p12)[cp11] chromosomal pattern (Figure 2). Therefore, including the present case, the male to female ratio is 11:4, resulting in an even lower male preponderance as is usually found in ordinary chromophilic/papillary RCC.

A remarkable finding in chromophilic/papillary RCC exhibiting the t(X;1) is that these tumors have a tendency to develop in children and young adults [6-9,120]. Normally, RCC is a disease of the eldery. The incidence increases with each decade of live, showing a peak incidence in the sixth decade [5]. Of the eleven cases of Xp11.2 chromophilic/papillary tumors, in which the age of the patient is provided, seven have an unusual youg age, varying from 1.5 to 29 years. Furthermore, these neoplasms also show a distinct morphology. Instead of the characteristic eosinophilic or basophilic cell type, the cells resemble large clear cells, due to the deposition of fat and glycogen [56,120,121].

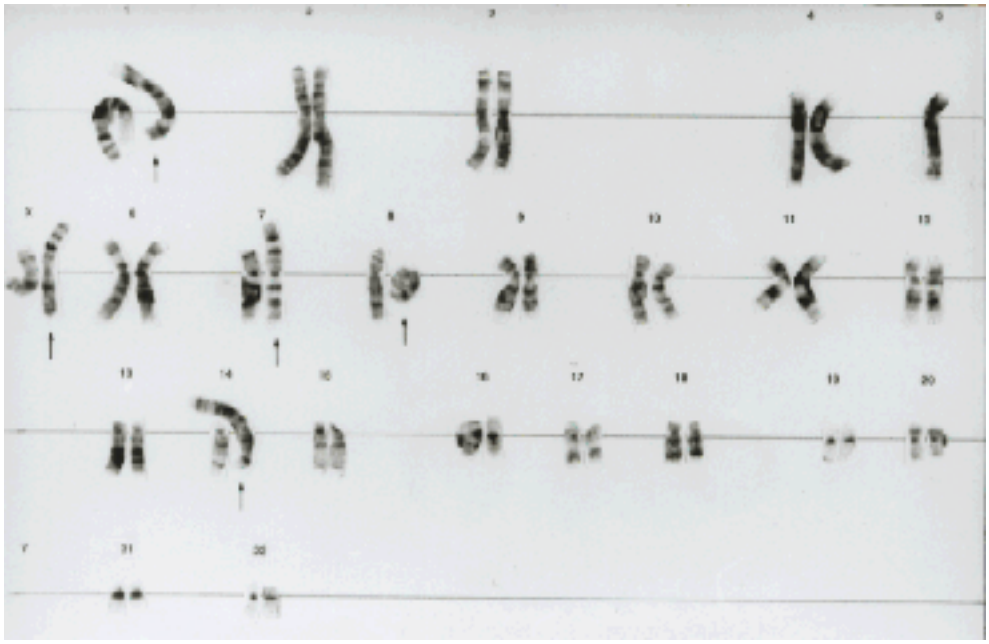

Figure 2: Representative karyotype of the present tumor, showing a 45,X,t(X;1)(p11;q21), -5,add(7)(p22),r(8)(p23;q24),der(14)t(5;14)(q11;p12) chromosomal pattern.

A papillary architecture, usually found in this RCC subtype, may not be predominant, resulting in a misdiagnosis of non papillary RCC, when the commonly accepted criteria of the WHO are applied. A diagnosis made on the specific cell type, as in the classification of Thoenes and Störkel [20,24] or the acceptance of genetic changes as diagnostic criteria, would be more appropiate, leading to a correct diagnosis of chromophilic RCC. Taken together, chromophilic RCC showing Xp11.2 translocations are distinct neoplasms, with a distinct morphologic appearance and age distribution, and a moderate male preponderance. Whether these tumors have a different biologic behavior has to be established by long term follow up studies.

CHAPTER 4

RENAL CELL CANCER OF THE COLLECTING DUCT SYSTEM

CHAPTER 4.1

CHROMOSOME CHANGES IN A METASTASIS OF A CHROMOPHOBE RENAL CELL TUMOR

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Cancer Genetics and Cytogenetics: submitted

ABSTRACT

Metastatic disease is a well known complicating factor in the treatment of renal cell cancer. Whereas radical nephrectomy usually is curative in cases of localized disease, no adequate treatment has been established for metastatic renal tumors. Identification of specific chromosome changes or genes responsible for metastatic behavior may lead to new strategies of treatment in the future. In this light we cytogenetically analyzed a metastasis of a chromophobe renal cell carcinoma arising in a 73-year-old male. The chromosomal pattern of the present case showed the extensive chromosome losses specific for the chromophobe subtype. Furthermore structural rearrangements involving chromosomes 1, 5, 12, 15, and 18 were observed. Whether or not (some of) these changes are important for the metastatic behavior of chromophobe renal cell tumors has to await further genetic studies on metastases of renal cancer.

Chromophobe renal cell cancer (RCC) is a distinct subtype of RCC, comprising approx. 4% of cases [5,20,24]. This entity was first described in humans by Thoenes et al. [124]. Chromophobe RCC is found to be histologically related to the intercalated cells of the collecting tubule, an origin they share with the benign oncocytomas [125]. The cells of chromophobe tumors are characterized by a pale, fine reticular cytoplasm, showing a positive reaction with Hale's acid iron colloíd stain. Numerous invaginated vesicles are observed in the cytoplasm, and a variable number of normal and morphologically altered mitochondria can be found. Cytogenetic and molecular genetic data on chromophobe RCC is limited, but almost all cases described thusfar are characterized by extensive nonrandom chromosome losses [60,61,71,126,127]. Telomeric associations and telomere shortning have also been observed [73]. Furthermore, molecular analysis of this subtype has revealed gross alterations of the mitochondrial DNA [54].

About 30% of all patients with RCC present with metastatic disease at intitial diagnosis [16,128], but different RCC subtypes have a different metastatic frequency. Metastases of clear cell RCC are frequently observed, whereas chromophobe RCC rarely

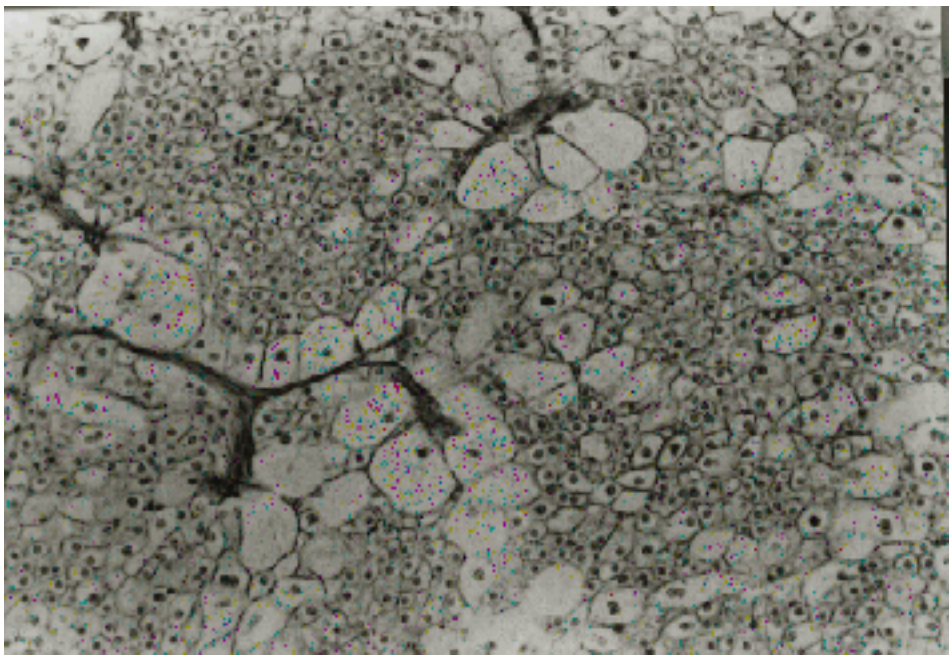

Figure 1: Histology of the chromophobe metastasis (H&E)

metastasizes. Papers dealing with the genetic constitution of RCC metastases are scarce, and only concern metastases of the clear cell type. In these cases a relation has been found between metastases and mutations of the *p53* gene [85], and loss of sequences at 9p [129] and 14q [26]. A few metastases of chromophobe RCC have been mentioned in the literature [5,130], but thusfar no cytogenetic data are available of chromophobe metastases.

We present the cytogenetic analysis of a chromophobe metastasis arising in a 73 year old male. The patient presented with a primary chromophobe tumor measuring 7 x 8 x 5 cm. This tumor had an orange to beige cut surface intermingled with some focal bleedings. Nodular tumor appearance was present and infiltration of the renal pelvis was observed. A metastasis, infiltrating the spleen, was removed from the abdominal wall. This lesion measured 11 x 9 x 9 cm, weight 350 g, and consisted of a solid tumor mass with focal necrosis and hemorrhages. On cut surface the specimen was beige to gray. Microscopic examination revealed a typical chromophobic tumor with a solid growth pattern. Large chromophobic cells were arranged close to the vascular interstice, smaller eosinophilic chromophobic cells were situated in the center of the tumor (Figure 1).

Cytogenetic analysis of a representative sample of the metastasis revealed a hypodiploid chromosome number in all 15 cells examined. The composite karyotype was: 33~37,X,-Y,-1,der(1)t(1;15)(q44;q13),-2,del(5)(q22),-6,-10,der(12)t(5;12))(q13;q22),-13,-15,add(15)(p11),-17,-18,add(18)(q22),-21,-22,+mar[cp15] (Figure 2). A few other cells showed a doubling this pattern.

As holds for primary chromophobe RCC described in the literature, in the present metastasis a low chromosome number was observed, with loss of chromosomes 1, 2, 6, 10, 13, 17, 18, 21, 22, and Y. Loss of chromosomes 9p and 14q, which have been associated

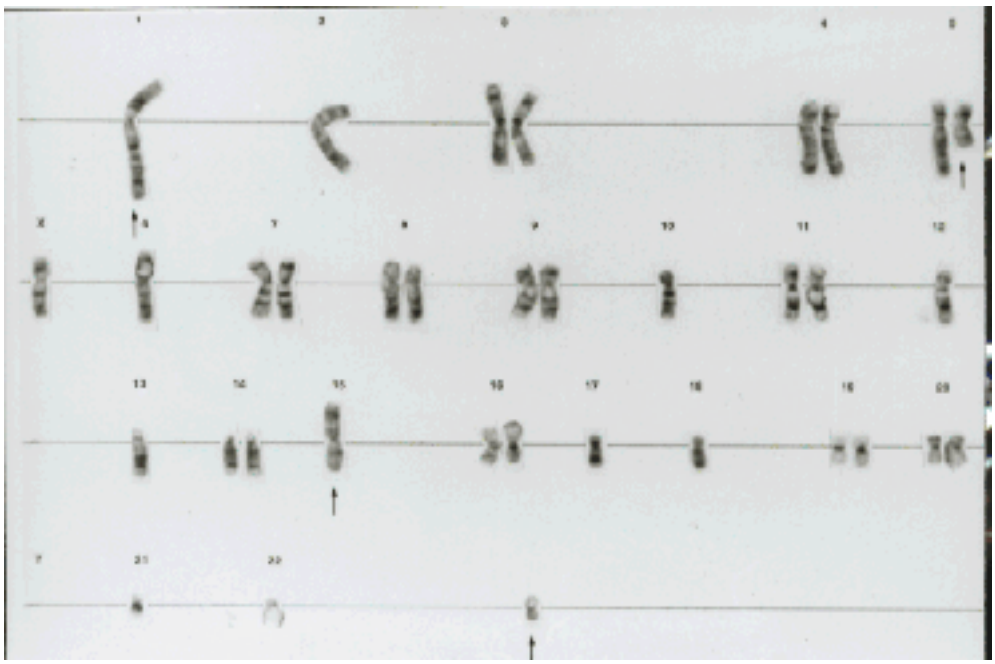

Figure 2a

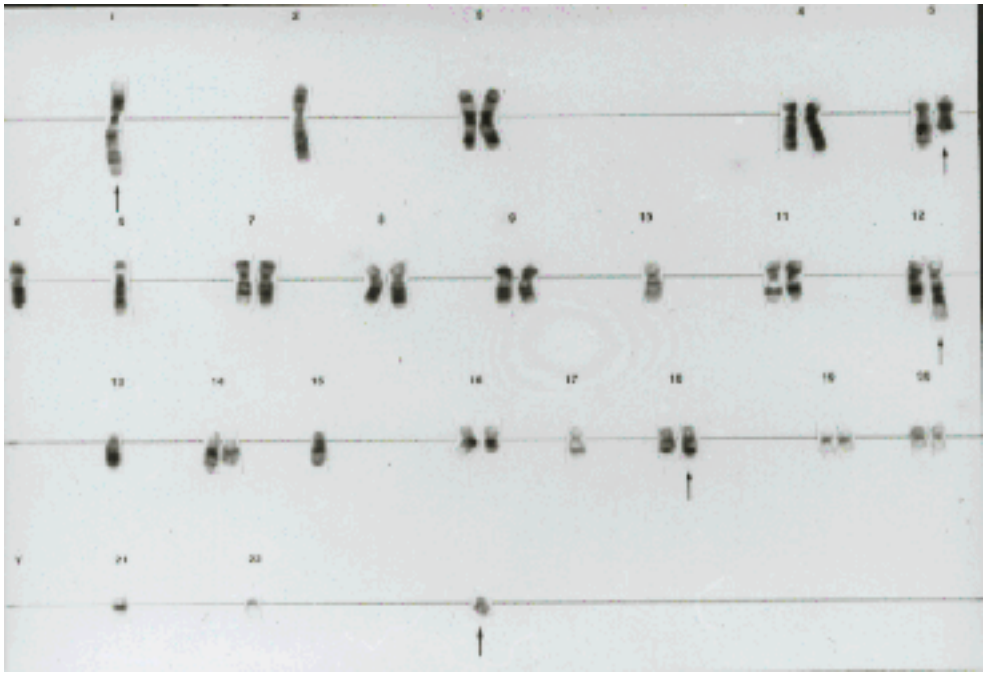

Figure 2b

Figure 2: Karyotypes of two of the metaphases of the metastasis. The composite karyotype of this case is described in the text.

with metastatic behavior in clear cell RCC, was not found in the chromophobe metastasis presented here. Unfortunately, no material was available for *p53* mutation analysis, but one copy of chromosome 17 was missing. However, the genetic changes associated with metastatic behavior in clear cell RCC might be different from those related to metastatic behavior in the chromophobe subtype.

In addition to the extensive chromosome losses mentioned above, in the present case strucural rearrangements involving chromosomes 1, 5, 12, 15, and 18 were observed . Structural changes of chromosomes 1 and 12 were also found in two chromophobe cell lines, derived from a grade II chromophobe RCC, pT3a pNX pMX [127]. These changes resulted in loss of regions 1q32-1qter and 12q13-12qter. In our case the regions 1q44-1qter and 12q13-12qter were lost, giving as common regions of deletion 1q44-1qter and 12q13-12qter. Alterations of the long arm of chromosome 5 are frequently observed in the clear cell subtype, resulting in gain of 5q sequences, mostly of the region 5q22-5qter [26]. Gain of 5q13-5q22 was observed in the present case. The other chromosome changes, i.e. add(15) and add(18), and involvement of chromosome 15 in the der(1) mentioned above, have not been described in the chromophobe subtype, nor have they been specifically associated with other RCC subtypes. Whether or not these changes are important in the development of RCC metastases in general, or more specific in metastases of the chromophobe subtype, has to be elucidated. Further studies on metastases of the different RCC subtypes may lead to the identification of specific chromosomal abnormalities and genes responsible for metastatic behavior in RCC. Subsequently this knowledge can serve as a tool in the development of new therapies in the future.

CHAPTER 4.2

INVOLVEMENT OF THE CHROMOSOMAL REGION 11q13 IN RENAL ONCOCYTOMA: A CASE REPORT AND LITERATURE REVIEW

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Cancer Genetics and Cytogenetics: in press

ABSTRACT

Renal oncocytomas comprise a cytogenetically heterogeneous group of tumors consisting potentially of cytogenetic distinguishable subgroups. Review of the literature revealed loss of chromosome 1 and Y as a possible anomaly for at least one subset of oncocytomas. The frequent finding of rearrangements involving chromosome 11 band q13 characterizes another subset of oncocytomas.
We report the cytogenetic and pathological features of a renal oncocytoma diagnosed in a 72-year-old woman and found a t(9:11)(p23;q13) as a consistent abnormality. This supports the idea that translocations involving 11q13 define a further subset of oncocytoma.

INTRODUCTION

Renal oncocytomas account for 5% to 10% of all renal tumors [70] and have been recognized as a distinct form of renal neoplasia with particular morphology and generally benign behavior. Although there are carcinomas with features resembling oncocytomas, these cases have to be excluded from the oncocytoma concept. True oncocytomas consists of large, well-differentiated eosinophilic granular cells. The oncocytic cell type expresses electronmicroscopical and immunohistochemical characteristics of the intercalated cell type A of the collecting duct and usually grows in an acinar pattern [20].
At the genetic level, renal oncocytomas are marked by alterations in their mitochondrial DNA and by lack of rearrangements of chromosome 3p, which seem to be a hallmark for the clear cell type of RCC [67,69,131].
Cytogenetic data on renal oncocytomas are sparse. In some cases combinations of -Y and -1 are described as common abnormality [70,71,132]. In other cases translocations involving 11q13 are observed, sometimes as the sole abnormality. These cases probably represent a second subset of oncocytomas [46,69,133,134].

We present a case of renal oncocytoma with a t(9;11)(p23;q13) and an additional dic(20;21)(p12;p13) and discuss our results in view of previously reported findings.

CASE REPORT

A 72-year-old woman was diagnosed in 1993 as having a kidney tumor. The surgically resected material consisted of a spherical mass, 3.5 cm in diameter. Histologic examination revealed the diagnosis of renal oncocytoma pT1,NX,MX. The tissue specimen of the primary renal oncocytoma was obtained from the Departments of Urology and Pathology, Johannes Gutenberg University, Mainz, Germany.

MATERIAL AND METHODS

Tumor and corresponding normal renal tissue specimens were minced and disaggregated with collagenase at a final concentration of 200 U/ml. Disaggregated cells were washed with RPMI 1640 medium supplemented with 20% FCS (PAA: Linz, Austria), 200mM L-Glutamine (Gibco: Paisley, Scotland) and Penicillin/Streptomycin in a final concentration of 5000 U/ml;5000 mg/ml, seeded in 80 cm^2 tissue-culture flasks (Nunc; Roskilde, Denmark) and cultured in a 5% carbon dioxide incubator at 37^oC in a humidified atmosphere for 6 days. Cultures were harvested and chromosome preparations were made using standard cytogenetic techniques. The chromosomes were GTG-banded and karyotypes described according to ISCN 1995 [135].

RESULTS

Of the 52 GTG-banded metaphases analyzed, 29 (56%) had a t(9;11)(p23;q13) as sole karyotypic abnormality. In 9 (17%) we found an additional dic(20;21)(p12;p13) A typical example is given in Figure 1. Nine cells presented loss of chromosome 11 in combination with two normal copies of chromosome 9, the remaining 5 cells showed a normal female karyotype, 46,XX without structural changes. Cytogenetic analysis of 15 cells of the corresponding normal tissue revealed a normal karyotype, 46,XX.

DISCUSSION

Oncocytomas of the kidney are tumors displaying unique gross microscopic and ultrastructural features. They account for 5 to 10 percent of all renal tumors [70]. Typically, renal oncocytomas exhibit a benign clinical behavior, but reports of bilateral cases, multiple tumors within the same kidney, occurrence of oncocytoma with contralateral renal cell carcinoma, and of extension into perinephric adipose tissue and colon exist [136]. There are discussions that some may possess malignant potential, but uncertainty comes up when these tumors are not strictly diagnosed.
Cytogenetic analysis of the present case showed a t(9;11)(p23;q13) as characteristic karyotypic change in 72% of the cells investigated. In 17% of the cells we found an additional dic(20;21)(p12;p13), 17% showed a 45,XX,-11 karyotype. We exclude the possibility of a constitutional rearrangement by the finding of a normal female karyotype in the corres*Table 1: Previously reported cytogenetic abnormalities in renal oncocytoma*

Reference	n	Summary of clonal abnormalities
Psihramis et al. [137]	1	46,X,-X,-3,+7,-14
Kovacs et al. [138]	1	45,X,-X,rcp(8;19),der(13)t(x;13)(q11;p13)/46,XY,del(1)(q21),der(13)t(1;13)(q21;q34)
Psihramis et al. [136]	1	44,X,-Y,-1
Miles et al. [139]	1	44,X,-Y,-1
Kovacs et al. [67]	1	45,XY,-19,-20,+mar
Walter et al. [134]	1	46,XY,t(9;11)(p23;q12)
Jordan et al. [140]	2	44,X,-Y,-1
Presti et al. [46]	1	46,XY,t(5;11)(q35;q13)
Crotty et al. [71]	2	44,X,-Y,-1
	1	45,XY,-22
	1	46,XY/45,X,dic(Y;22)(q11.2;p13)
	1	46,XY/47,XY,+7/45,XY,-9,-20,+mar
Meloni et al. [70]	1	44,X,-Y,-1
Dobin et al. [132]	1	40,X,-X,der(1)inv(1)(pter→1p36.1::1p13.3→1q12::1p36.2→1p21::?::1q22→1qter, -2,-4,-5,-6,-17
	1	47,XY,+12
	1	43,X,-Y,-1,-15,+16,-22
	1	45,XX,dic(14;17)(p11;p13)
Füzesi et al. [133]	1	46,XX,der(11)ins(9;11)(p23;q13q23)ins(20;11)(q13;q23q25),del(22)(q13)
Van den Berg et al. [69]	2	46,XY,t(5;11)(q35;q13)
	1	43~47,X,-Y,+3,+14
	1	44~49,XX,+7
	1	45,X,-Y
	1	40~41,X,-Y,-1,der(9)t(9;?11)(p24;q14),-11,-14,add(19)(q13),-21,-22,+r(6)
	1	81~90,XXXX,-1,-10,tas(15;22)(p13;p13)
present case	1	46,XX,t(9;11)(p23;q13)/45,XX,idem,dic(20;21)(p12;p13)/45,XX,-11

ponding renal tissues.

In the literature over 30 cases of renal oncocytoma have been cytogenetically characterized so far (for review, see Table 1). Crotty et al., Dobin et al., Psihramis et al., Meloni et al. and Kovacs et al. [70,71,132,136,138], described coincident loss of the Y chromosome and chromosome 1 as characteristic anomaly in renal oncocytoma.

In 1989, Walter et al. [134] were the first who described a t(9;11)(p23;q12) translocation as the only karyotypic change in a renal oncocytoma. They interpreted this anomaly as primary, possibly specific change in this type of tumor. Presti et al. [46] analyzed in 1991

a series of renal cell carcinomas including five renal oncocytomas. They also described a translocation t(5;11)(q35;q13) as characteristic structural rearrangement. Van den Berg et al. [69] analyzed in 1993 a series of nine renal oncocytomas. They found a t(5;11)(q35;q13) as the sole chromosomal anomaly in a subset of two oncocytomas.
Structural abnormalities affecting chromosomal band 11q13 are consistent features in a number of malignant disorders with a different histogenetic origin including renal cell tumors [141]. Chromosome 11q13 harbors a number of proven and potential oncogenes, including growth factor genes, a growth factor receptor gene, the cell cycle regulatory gene CCND1, and the oncogene EMS1.

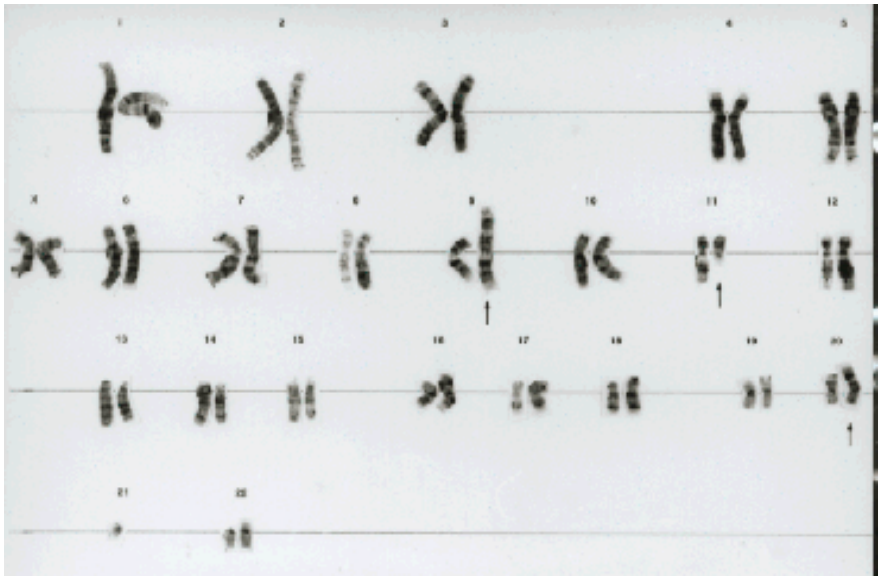

Figure 1: Karyotype of a tumor cell with 45,XX,t(9;11)(p23;q13),dic(20;21)(p12;p13).

Oncocytomas contain numerous mitochondria and are marked by alteration in their mitochondrial DNA [67,131]. Notably, mitochondrial proteins are encoded in the vicinity of the rearranged sites on chromosome 1, 11, and 20 [142-144]. This suggests that the development of oncocytomas might be determined by more than a single chromosomal site and that mitochondrial enzymes play an important role in the tumorigenesis of oncocytoma. Loss of chromosomes 1 and Y might be characteristic chromosomal abberations of one subgroup of oncocytoma. Alteration in chromosomal band 11q13 may be the primary change in another subgroup and rearrangements including chromosome 20 may be a further step in the development of these tumors.
However, more cytogenetic, molecular and FISH- analysis are necessary to confirm the idea of at least two different subgroups of oncocytoma and to find possible candidate genes in the chromsomal bands involved.

CHAPTER 4.3

ARE ALL RENAL ONCOCYTOMAS, RENAL ONCOCYTOMAS?
Renal oncocytoma showing a t(5;12;11)(q22;p12;q13q24), and a literature review

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The International Journal of Cancer: submitted

ABSTRACT

Renal oncocytomas reveal a considerable (cyto)genetic heterogeneity. At least two genetic subsets are presently recognized, characterized by 1) translocations involving breakpoint 11q13 and 2) the combined loss of chromosomes 1 and X/Y. We present a case of oncocytoma, revealing a threeway translocation involving breakpoint 11q13. Using FISH, the 11q13 breakpoint of the present case proved to be slightly different from the one observed previously in three cases of renal oncocytoma. Whether or not the 11q13 breakpoint observed in our case resides in or near another gene has to be elucidated.
The malignant potential of renal oncocytomas is still a matter of debate, but the genetic constitution of these neoplasms may hold key information about their biologic behavior. Comparison of the chromosomal patterns of renal oncocytomas and chromophobe carcinomas, shows that loss of chromosomes 1 and X/Y is shared by both tumor types. A literature review reveals that the close relationship between renal oncocytomas and chromophobe renal cell carcinomas, is further substantiated by the fact that both subtypes share a number of morphologic and histogenetic features. Hence, we propose that renal oncocytomas characterized by loss of chromosomes 1 and X/Y may be chromophobe adenomas. Additional whole chromosome losses may result in progression to a chromophobe carcinoma stage. The observations suggest that, possibly, not all renal oncocytomas are oncocytomas, explaining the malignant behavior occaisionally observed in these neoplasms.

INTRODUCTION

Renal oncocytoma is a distinct subtype of renal cell cancer (RCC), comprising about 4% of cases, in which the male to female ratio is 2.5:1. It is an essentially benign neoplasm and clinically most of these tumors are discovered incidentally [5]. However, malignant renal oncocytomas have

been described, indicating that at least some of these neoplasms have malignant potential [136,145]. Histologically, renal oncocytomas consist of enlarged cells with small, centrally located, nuclei. A prominent feature of renal oncocytic cells is the accumulation of enlarged mitochondria in the cytoplasm. Different authors have observed specific changes in the mitochondrial DNA of renal oncocytoma [26,131]. The frequent finding of telomeric associations, reflecting a specific type of chromosome instability, is another characteristic property [73].
Cytogenetic analysis of renal oncocytomas has revealed a variety of chromosomal patterns, suggesting the existence of distinct subsets [26,69-71,132,136,146]. At least two genetically defined subsets seem to emerge, characterized by 1) the combined loss of chromosomes 1 and X/Y and 2) translocations involving chromosome 11 with breakpoint 11q13. Recently Sinke et al [147] mapped this oncocytoma specific 11q13 breakpoint in two t(5;11)-positive renal oncocytomas, between D11S443/D11S146 and the BCL1 locus. The 11q13 breakpoint of a third case, revealing a t(9;11)(p23;q13) was found to be located within the same region (Geurts van Kessel, personal communication), suggesting that in the t(5;11)- and the t(9;11)-positive renal oncocytomas the same gene at chromosome 11 may be involved in the development of these neoplasms. The remaining cases of oncocytoma display mixed populations of cells with normal and abnormal karyotypes, which fail to show any cytogenetic similarity [26].

Since little is known about the genetic changes responsible for the development of renal oncocytomas, additional studies concerning the genetic constitution of these neoplasms are mandatory. Furthermore, the cases of renal oncocytoma showing local or distant metastases might have genetic changes distinct from those showing a benign behavior. In these cases, the genetic constitution may prove to be a valuable diagnostic and prognostic tool. Hence, we analyzed a case of renal oncocytoma using cytogenetic techniques and FISH, and compared the observed findings with data obtained from the literature. In addition, we aimed to find a relation between the biologic behavior of renal oncocytomas and their genetic profile. In this light we comment on a possible, genetically defined, distinction between real oncocytomas, chromophobe adenomas, and chromophobe carcinomas.

CASE HISTORY

A 63 year old male presented with a solid renal mass. Tumornephrectomy revealed a sharply demarcated tumor nodule measuring 5x5x4.5 cm with extension into the hilar fatty tissue. On cut surface the tumor was light brown. Microscopic examination showed large eosinophilic, granular tumor cells with increase of double nuclei and focal extremly pleomorphic nuclei arranged in a mostly solid acinar and partially tubulocystic architecture. No peritumoral fibrous pseudocapsule was observed and no lymphocytic infiltration was seen. The tumor was diagnosed as a renal oncocytoma (Figure 1).

MATERIAL AND METHODS

Fresh representative samples of the tumor and adjacent nontumorous renal tissue were submitted for cytogenetic investigation. Culteres were maintained for 13 days in RPMI 1640 supplemented with FCS (16%), glutamine and antibiotics.

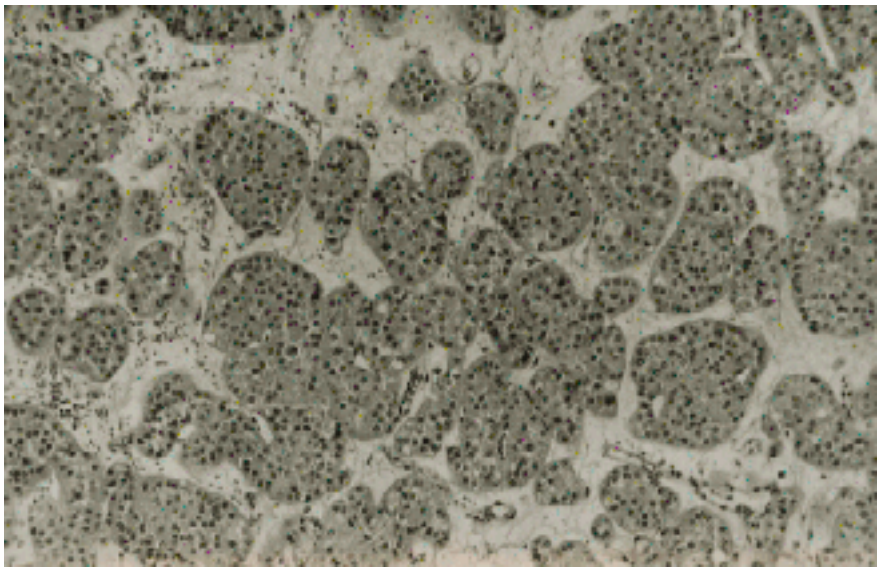


Figure 1: Histology of the present oncocytoma (H&E 91x)

The cultures were harvested and chromosome preparations were made according to standard cytogenetic techniques. The chromosomes were G-banded using pancreatin and karyotypes were described according to the ISCN'95 guidelines for cancer cytogenetics (Figure 2).
For FISH studies specific libraries for chromosomes 5, 11, and 12 (commercially available from ONCOR) were used. To identify chromosome 5, a hybridization with CEPH YAC 933A7 was carried out. Identification of chromosome 12 was done using probe pαH8. For labeling of the YAC and pα12H8, the Bio-Nick Labeling System (Gibco BRL) was used. In situ hybridization was carried out essentially according to the manufacturer's protocol (Figure 3). Cosmids CcI11-44 (D11S443), BCl1-cl9, and Ccl11-59 (D11S146) were used to further define the breakpoint at 11q13. These experiments were carried out as described previously [147]. Cosmid cCLGW454 (D11S688) located at 11p15, served to identify chromosome 11.

RESULTS

Cytogenetic and FISH analysis of the present case resulted in a 46,XY,der(1)t(1;8)(q43;q11.1),t(5;11;12)(q22;p12;q13q24).ish(wcp5+,933A7+,wcp11+,wcp12+;wcp11+,D11S688+,D11S443+,D11S146+,BCl1cl9+;wcp5+,wcp12+,pα12H8+),add(19)(p13)[cp10] chromosomal pattern. A karyotype of one of the metaphases is given in Figure 2. FISH results defining the threeway translocation are seen in Figure 3, showing in 3a) a paint for chromosome 11 (arrows) and YAC 933A7 (arrowheads), in 3b) a paint for chromosome 12 (arrows) in conjunction with YAC933A7 arrowheads), and in 3c) a paint for chromosome 5 (arrows) in conjunction with pα12H8 (arrowheads). Cytogenetic analysis of ten cells of the nontumorous kidney tissue revealed clonal loss of the Y chromosome.

DISCUSSION

Translocations involving chromosome 11 at 11q13 are a recurrent finding in a subset of renal oncocytoma (see for review [148]). In these tumors, chromosome 11 is specifically translocated to either chromosome 5 or 9. The genes involved in the oncocytoma-specific 11q13-translocations are not yet known, but recently, the 11q13 breakpoint has been mapped between D11S443/D11S146 and the BCL1 locus [147].

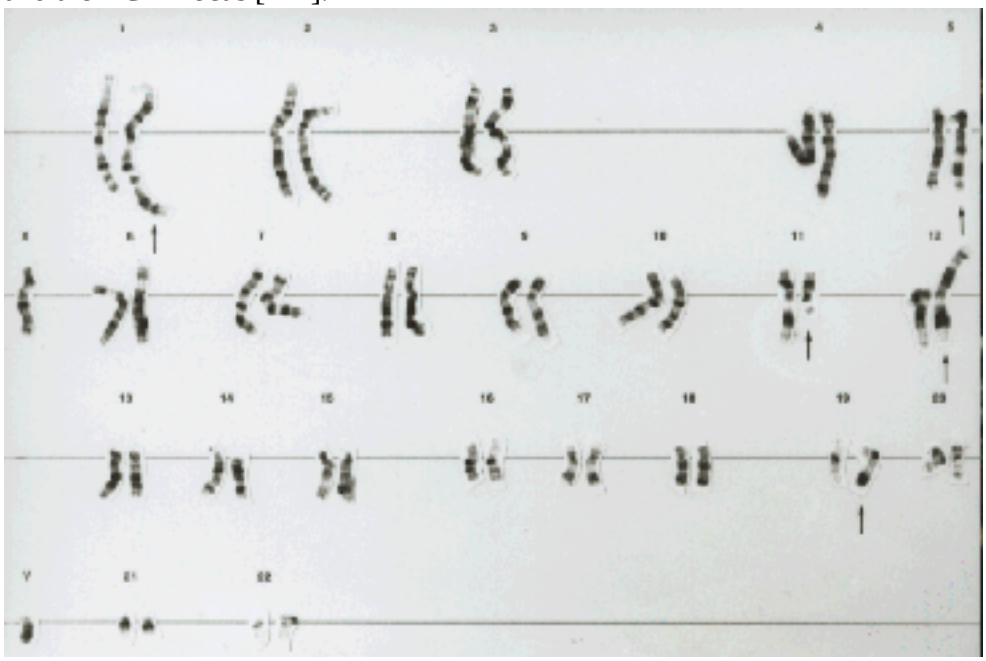


Figure 2: A representative karyotype of the present case. Karyotype description is given in the text.

Cytogenetic analysis and FISH of the present case revealed an apparently balanced translocation between chromosomes 5, 11, and 12 in all cells examined. The breakpoint at chromosome 5 clearly differed from that observed in the t(5;11)-positive oncocytomas published thusfar. Surprisingly, the breakpoint at chromosome 11q13, appearing similar at a cytogenetic level, proved to be (slightly) different using FISH. Cosmid BCL1-cl9, found to be located distal to the 11q13 breakpoint in the t(5;11)- and t(9;11)-positive oncocytomas, was located proximal to the 11q13 breakpoint of the present case. Whether or not the latter breakpoint resides in or near another gene has to be elucidated. Both the der(1)t(1;8)(q43;q11.1) and the add(19)(q13) were present in four of the ten cells analyzed. The der(1) resulted in loss of a small segment of chromosome 1 and gain of almost the whole 8q arm. Loss of chromosome 1 sequences has been described in renal oncocytomas [70,71,136,149,150]. Usually the whole chromosome 1 is missing in these cases, but recent data indicate that especially loss of 1p is important [149]. In most cases, loss of chromosome 1 sequences occurs in combination with loss of the X or Y chromosome. In the present case retention of the Y chromosome was observed, and loss of chromosome 1 sequences involved the region 1q43-qter, and not 1p. Gain of chromosome 8 has not been associated with renal oncocytomas thusfar. An add(19)(q13) has been described in one of the cases of oncocytoma published by Van den Berg et al [69]. In this case also loss of chromosomes 1 and Y and a t(9;?11) was observed but

the latter showed different breakpoints than the t(9;11) thought to be specific for renal oncocytomas. Whether or not the present case belongs to the subgroup characterized by 11q13 translocations has to await the identification of the genes responsible for the development of this particular group of renal oncocytomas.

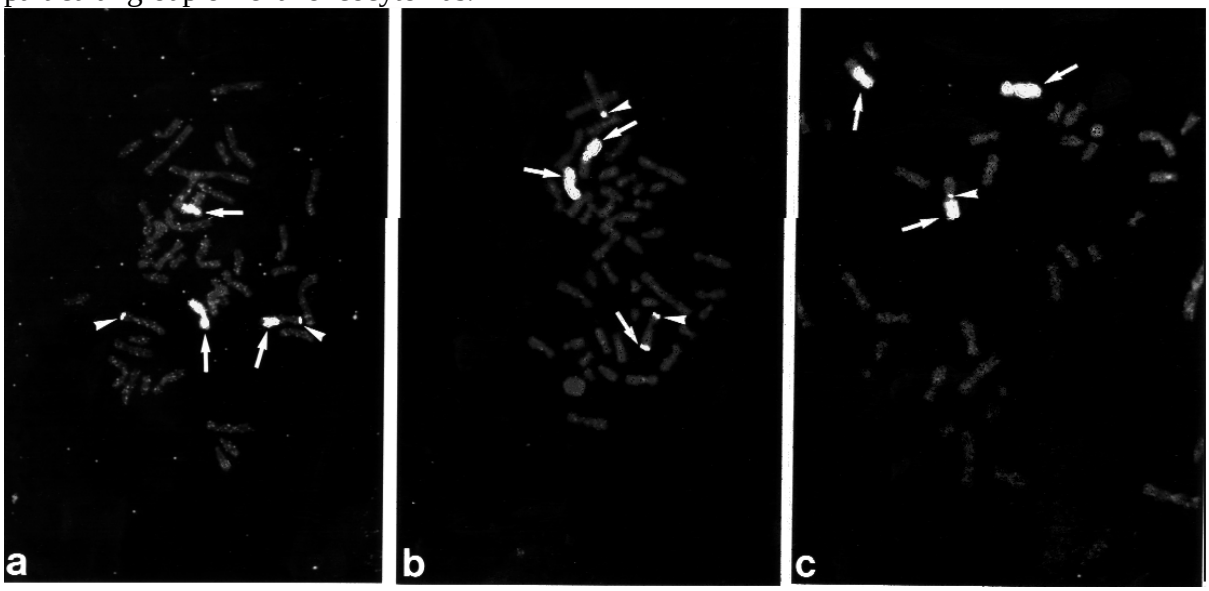


Figure 3: FISH on metaphase spreads of the present case using a) a paint for chromosome 11 (arrows) in conjunction with YAC 933A7 (arrowheads), b) a paint for chromosome 12 (arrows) in conjunction with YAC 933A7 (arrowheads) and c) a paint for chromosome 5 (arrows) in conjunction with pa12H8 (arrowheads) defining the threeway translocation between chromsomes 5, 11, and 12.

A major concern about renal oncocytoma is its malignant potential. Although the majority of reported cases behave in a benign fashion, evidence is accumulating that a small subset of oncocytomas are malignant [5,136,145]. The malignant potential of the latter might be associated with distinct genetic changes, the establishment of which might be important for diagnosis and prognosis. Unfortunately, papers presenting the combined data of both cytogenetic results and clinical follow up are scarce, and therefore provide no evidence for a strict relation between genetic constitution and clinical outcome in renal oncocytomas. Nevertheless, comparison of the genetic profile of renal oncocytomas with other, malignant, RCC subtypes, reveales that a subset of oncocytomas as well as chromophobe renal cell carcinomas, consistently show loss of chromosomes 1 and X/Y. In addition to loss of chromosomes 1 and X/Y, chromophobe carcinomas are characterized by the combined loss of chromosomes 2, 6, 10, 13, 17, and 21 [61,108,126]. Morphological and immunohistochemical data support the assumption of a close relationship between renal oncocytomas and chromophobe RCC [125,151,152]. Both tumor types find their origin in the intercalated cells of the collecting tubules, and both express carbonic anhydrase C. Other common features are the finding of telomere shortening, telomeric associations, and mitochondrial DNA changes [26,73,131,153]. In view of the above mentioned findings, we propose that renal oncocytomas characterized by the combined loss of chromosomes 1 and X/Y, may be

chromophobe adenomas, which can progress towards a carcinoma stage through additional chromosome losses involving chromosomes 2, 6, 10, 13, 17, and 21, and thus may be potentially malignant. Evidence substantiating this proposed oncogenetic pathway are 1) the finding of an oncocytoma containing chromophobe cell nests [151], pointing to a possible transition between both tumor types, 2) the cytogenetic analysis of a case of oncocytoma revealing multiple chromosome losses, among which chromosomes 1, 2, 6, 17, 21, and Y [154], and 3) LOH studies, showing loss of chromosome regions 1p, 2pq, 13q, 17pq, Xpq, in one case of oncocytoma, and loss of 1pq, 14q, and 21q in another [149].

Clinical follow up is mandatory to draw conclusions concerning the above proposed oncogenetic pathway. The only case of malignant oncocytoma of which cytogenetic data has been provided, revealed a 44,X,-Y,-1 chromosomal pattern [136]. This finding is in agreement with a proposed malignant potential of tumors exhibiting loss of chromosomes 1 and X/Y, as mentioned above.

Clinical follow up has not been provided for the cases with 11q13 translocations published thusfar, but the two patients with t(5;11)-positive oncocytomas published by us [69] are presently alive without evidence of disease after 46 and 96 months respectively. In addition, no (5;11) or (9;11) translocations have been described in chromophobe carcinomas, nor in other RCC subtypes, suggesting that no relationship exists between chromophobe or other renal cell carcinomas and this subset of renal oncocytoma.

Taken together, (cyto) genetic analysis of renal oncocytomas show that these neoplasms may be even more heterogeneous than assumed, since the 11q13 translocation breakpoints observed as identical with conventional cytogenetic techniques may be different at the molecular level. The biologic implications of these findings are presently unknown, and whether or not a different gene at 11q13 is involved in our case has to be elucidated. In addition, renal oncocytomas displaying a combined loss of chromosomes 1 and X/Y may well be chromophobe adenomas. Progression towards a carcinoma stage in these neoplasms might occur through additional chromosome losses, explaining the malignant potential occasionally observed in renal oncocytomas. The oncocytomas showing translocations involving 11q13, and not loss of chromosomes 1 and X/Y, may be "true" oncocytomas, behaving invariably benign. Long term follow up studies, combined with the genetic changes present in the different subsets of renal oncocytomas are mandatory to elucidate this matter.

CHAPTER 5

5. SUMMARY AND GENERAL DISCUSSION

CHAPTER 5

SUMMARY AND GENERAL DISCUSSION

The aim of any histopathological classification is to use morphological criteria to identify disease states which are biologically distinct, the recognition of which are of clinical value [31]. Different classification systems are currently in use for renal cell cancer (RCC) but their criteria are not always conclusive. This is not surprising since renal cancers display a heterogeneous morphology and their phenotype may change dramatically during progression [27]. The considerable diversity of RCC stresses the need for a more extended and refined classification than the commonly used WHO classification, which divides renal tumors into adenomas, carcinomas, and others, and does not allow extensive subtyping [2]. In 1986 Thoenes and Störkel proposed a new morphological classification for RCC, in which five different RCC subtypes are recognized, related to the basic cell types of the nephron from which they are derived [20]. Clear cell and chromophilic RCC arise from cells of the proximal part of the nephron, renal oncocytomas and chromophobe RCC find their origin in the intercalated cells of the collecting tubule, and Duct Bellini carcinomas are associated with the principle cells of the collecting tubule. Variants of the basic cell types, characterized by an accumulation of mitochondria (eosinophilic variants), are also mentioned. Three growth patterns: compact, tubulo-papillary and cystic are distinguished. Principally, in a given tumor, all growth patterns can occur simultaneously, but generally one of them predominates. There are relationships to the cell types, although not exclusive: clear cell and chromophobe RCC is predominantly related to compact growth, chromophilic RCC to tubulo papillary growth, renal oncocytoma to acinar growth, and Duct Bellini carcinoma to both compact and tubulopapillary growth.

Since it is generally accepted that genetic alterations, stable during cell division, are the fundamental cause of neoplastic transformation, the genetic profile of different subtypes of RCC may hold key information about oncogenesis, progression, and pathogenetic relationships of tumor types. Evidence is accumulating that RCC can be divided into genetically distinct entities. The concept of a genetic classification for renal neoplasms has been advocated by Kovacs and by us for several years [23,25-28]. A great advantage of a genetic approach is that, contrary to tumor phenotype, in general primary genetic changes are constant during tumor development and progression. Once present, these changes mark all descendent cells. Furthermore, distinct genetic changes may be associated with tumor progression. Assessment of the genetic constitution might prove to be a powerful diagnostic and prognostic tool in the clinical management of renal cancer. Establishment of the genetic constitution of different RCC subtypes might contribute to, and refine a morphological subtyping, but might also contradict existing morphological classifications and grading systems, possibly leading to a revised classification.

Combining the morphologic and genetic data of more than 200 cases of renal cancer, some of which have been discussed in the present thesis, and data extracted from the literature, we present an oncogenetic model for the development and progression of the heterogeneous group of RCC, depicted in Figure 1. Morphologic features are determined according to the classification proposed by Thoenes and Störkel [20,24]. In addition to morphological subtyping, we divided the different

RCC subtypes into two main groups, related to the embryologically different tissues of origin of the renal tubular system (see chapter 1). In Figure 1, the mature renal tubular system is represented as a white bar in which the proximal and distal tubule and the collecting tubule are recognized. The collecting tubule is separated from the proximal and distal tubule by a grey line, reflecting their different embryonal origin, i.e. the mesonephros and the metanephros, respectively. The next horizontal lane indicates the adenoma stage of the different subtypes, related to their presumed cell of origin. Each subtype is characterized by a distinct combination of genetic changes, reflecting its unique developmental pathway. In vertical direction, progression to a carcinoma stage, and subsequently progression to carcinomas of higher grade and stage, including sarcomatoid transformation is shown. The genetic changes associated with each of these processes are given. If determined, chromosome arms p (short) and q (long) are mentioned and the breakpoint designation, according to the recommendations of the International Standing Committee on Human Cytogenetic Nomenclature, is provided. The morphologic and genetic features of each of the five subtypes, in relation to histogenesis, pathogenesis, oncogenesis and classification, are discussed in detail in the sections below. Furthermore the correlations and differences between morphological and genetic subtyping will be emphasized.

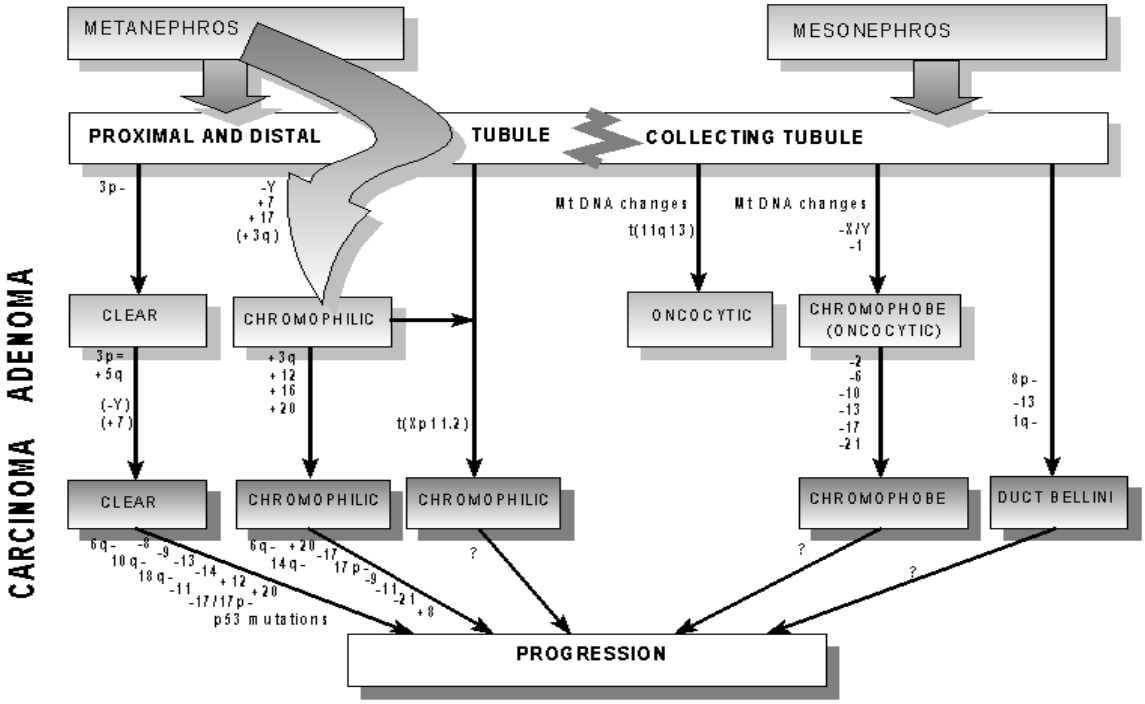
Genetic changes associated with normal kidney tissue:

Increasing evidence exists on the presence of clonal, mostly numerical, chromosome changes in apparently normal kidney tissue from patients with a normal constitutional karyotype [32,34,35,155,156]. Loss of the Y chromosome and trisomy of chromosomes 7 and 10 have frequently been described. These changes are not an in vitro artefact and are independent of the length of cell culture [32]. Trisomy 7 and 10 and loss of the Y chromosome are also observed in renal cell tumors, raising the question whether these cells in the kidney have some inherent propensity for neoplastic transformation. Some authors have proposed that trisomy 7 represents the first oncogenetic step in the development of renal tumors. However, several tumors genetically showing 3p deletions, do not have a trisomy 7. It has been shown that, in general, approx. 10% of kidney cells have trisomy 7 and neoplastic transformation occurs at similar frequencies in cells with and without trisomy 7 [157]. The presence of clonal and non clonal aberrations in apparantly normal kidney tissue merely indicates a chromosome instability pattern or mosaicism, and this condition should not be considered as strictly neoplastic.

Renal cell cancer of the clear cell and chromophilic type:

Clear cell RCC also referred to as non papillary RCC is the most common form of RCC, comprising approximately 70-75% of cases. They show a male preponderance of approximately 2:1. Hereditary as well as sporadic cases of clear cell RCC are found. Hereditary RCC is characterized by the appearance of multiple and bilateral tumors and an early age of onset.

Figure 1: Proposed oncogenetic model for renal cell cancer



The Von Hippel-Lindau (VHL) cancer syndrome is the most common form of hereditary RCC [158]. Retinal, cerebellar and spinal haemangioblastomas, RCC, pheochromocytoma, and renal, pancreatic and epididymal cysts are specific findings associated with VHL disease. Hereditary RCC is also found in families with a constitutional balanced translocation involving chromosome 3 [159]. Sporadic cases usually present as solitary tumors. The distinction between clear cell adenomas and clear cell carcinomas is usually made on size, but the existence of clear cell adenomas is a debatable field. Some authors have proposed that clear cell tumors are malignant, regardless of their size [31]. The tumor mass of clear cell RCC is multicolored, with a predominantly yellow cut surface, in which white or gray foci are seen. Mostly the tumor cells show a solid growth pattern, but in a few cases a cystic appearance is seen. The cytoplasm is clear, due to an intensive intracytoplasmic accumulation of glycogen and lipids. The nuclei are condensed and hyperchromatic in well differentiated tumor cells, whereas in less differentiated tumor cells, polymorphic nuclei and prominent nucleoli appear. Other features of a higher grade in clear cell RCC are an increased cytoplasmic granularity or eosinophilia due to the augmentation of mitochondria. The latter can be found either in the vicinity of the nucleus or more or less diffusely distributed within the cytoplasm.

Loss of sequences of the short arm of chromosome 3 are a characteristic finding in hereditary as well as sporadic cases of clear cell RCC. In the dominantly inherited von Hippel-Lindau (VHL) cancer syndrome, the *VHL* gene, assigned to 3p25, is mutated in the germ line and in renal cell tumors of affected family members [158]. In families with a constitutional balanced translocation, the breakpoints of chromosome 3p in the t(3;6) and t(3;8) are 3p13 and 3p14.2,

respectively. In tumor tissue of translocation carriers the derivative chromosome containing the distal 3p segment is consistently lost.
In sporadic cases the *VHL*-gene is mutated in 57% of cases, implying a significant but not exclusive role for this gene in the development of sporadic clear cell RCC [158]. Other regions at 3p frequently found to be lost are 3p12-14 and 3p21. Recent findings indicate that loss of at least two of the regions mentioned above are necessary for kidney cells to develop into clear cell carcinomas, and that loss of 3p21 is always involved [44]. In Figure 1 this double loss is pointed out as 3p=. Tumors showing loss of either 3p12-14 or 3p25, depicted as 3p- in Figure 1, should be designated clear cell adenomas [44]. Although loss of 3p sequences sometimes occurs as the sole genetic change, and thus may be sufficient for the initiation of clear cell RCC development, it is usually accompanied by other changes. The second most common abnormality observed in clear cell RCC is gain of (part of) chromosome 5 [26]. Trisomy 5 or partial trisomy of the smallest overlapping region (5q22-qter) occurs in 50% of cases. In several clear cell tumors trisomy of the 5q segment arises concurrent with loss of 3p sequences by the formation of an unbalanced translocation between chromosomes 3 and 5 [25]. Loss of the Y chromosome and trisomy 7 are also frequent findings, but they appear in similar frequencies in normal kidney tissue. Therefore the role of these chromosome changes in the development and/or progression of clear cell RCC is debatable. For this reason, trisomy 7 and loss of the Y chromosome are placed between brackets in Figure 1. Tumorprogression in clear cell RCC has been associated with a number of genetic changes. Loss of chromosome 14 has been associated with higher grade and stage in several studies [25,26,108,160,161]. A relation has been found between loss of 14q and increased genetic instability, thus facilitating the formation of additional chromosome changes [104]. Other changes involved in tumor progression in the clear cell subtype are loss of 6q, 8(p), 9, 10q, 11, 13, 17(p), and 18q, and gain of chromosomes 12 and 20 [26,48,76,161-163]. Two linked structural changes on 17p, being allelic loss and mutations of the *p53* gene, assigned to 17p, generally occur late in progression [85,164,165]. Mutations of te *p53* gene have also been associated with sarcomatoid transformation, an ultimate form of tumorprogression in renal cancer (see chapter 3.1.3)

Chromophilic RCC, often referred to as papillary RCC, comprise 10-15% of RCC cases. They show a strong male preponderance. The male to female ratio is approximately 8:1. Familial cases of chromophilic or papillary RCC have been described [89,155], but the cause of the familial appearance of this RCC subtype is not yet known. As in hereditary clear cell RCC, these tumors are characterized by a frequent multiple and bilateral appearance and an early age of onset. Sporadic cases of chromophilic RCC also may present as multiple/bilateral tumors (see chapter 3.12).

Chromophilic RCC can be divided into adenomas and carcinomas, in fact most adenomas of the kidney are of the chromophilic/papillary type. It may be difficult to distinguish chromophilic adenomas from carcinomas on histological grounds, and, as clear cell adenomas, they are usually diagnosed on their size. Chromophilic adenomas tend to be beige- to white colored small tumor masses (usually less than 3 cm). In chromophilic carcinomas an extensive greasy- brown colored central necrosis resulting from consecutive hemorrhages is frequently seen. The tumor cells exhibit centrally located small nuclei and the cytoplasm is covered with a few organelles only, especially endoplasmatic reticulum. As a rule the tumor cells are arranged in a (tubulo) papillary architecture, becoming solid in undifferentiated areas. In the latter, polymorphic nuclei with prominent nucleoli

and an eosinophilic or granular cytoplasm, due to an accumulation of mitochondria are seen. In rare instances chromophilic cell types contain fat and glycogen, resembling clear cells [20]. In these cases a papillary growth pattern may not be predominant.

Most chromophilic/papillary renal cell tumors are characterized by a unique combination of autosomal trisomies, in which trisomy 17 is the most consistent finding (see chapter 3.1.1). Adenomas specifically show a -Y,+7,+17 chromosomal pattern. In some cases also trisomy of chromosome 3(q) has been observed, but this might be an early reflection of malignant transformation [26]. In Figure 1 this is pointed out by (+3q). Subsequent gain of chromosomes 12, 16, and/or 20 marks the transition to a chromophilic/papillary carcinoma. The combined trisomy of chromosomes 7 and 17 as the sole autosomal change can be found in small as well as large tumors, whereas small tumors can have additional changes suggestive for malignant transformation. Therefore genetic analysis is mandatory in distinguishing chromophilic/papillary adenomas from carcinomas. Loss of the Y chromosome is observed in 85-93% of chromophilic/papillary tumors [75,166]. The high incidence of Y chromosome loss in this subtype, combined with the strong male preponderance, suggests that loss of specific sequences harboured on the Y chromosome probably is important in the development of this subtype. The incidence of -Y (85-93%) and trisomy 7 (87%) [75] is substantially higher than observed in appearantly normal kidney tissue, and therefore are considered essential steps in the oncogenesis of chromophilic/papillary RCC.

A multiple appearance has been associated with both familial and sporadic cases of chromophilic/papillary RCC. Cytogenetically, no differences are observed between hereditary and sporadic chromophilic/papillary tumors. Both show the characteristic combination of autosomal trisomies mentioned above. In a recent study, however, we demonstrated by chromosome 7 and 17 allelic imbalance studies, that differences do exist between the two. Sporadic cases of multiple chromophilic/papillary RCC show imbalance of the same chromosome 17 alleles in different tumors. Similar results have been published by Kovacs et al. [26]. It is highly unlikely that the same chromosome 17 is duplicated in so many tumors by chance. Furthermore, since most of the encountered tumors are adenomas, which have no metastatic potential, they cannot represent micrometastases. Therefore we propose that the first oncogenetic step in the development of sporadic chromophilic/papillary RCC occurs during kidney development as is discussed in chapter 3.1.2. In our model (Figure 1) the embryonal origin of these tumors is indicated by the big arrow, omitting the mature renal tubular stage. In the same survey, we found that in familial cases of chromophilic/papillary RCC, different alleles are randomly involved in chromosome 17 imbalance, suggesting that trisomy 17 is not the first oncogenetic step in the development of familial tumors (see chapter 3.1.2). At present we have no clue about the nature and localization of the inherited genetic defect responsible for the predisposition to develop chromophilic/papillary tumors in these families, and whether or not a similar genetic change also precedes gain of chromosome 17 in sporadic cases. Therefore, familial and sporadic chromophilic/papillary RCC are not mentioned as separate entities in Figure 1.

Little is known about the genetic basis of tumor progression in chromophilic/papillary carcinomas. In a recent paper (see chapter 2.1.1), we observed that tumorprogression is related to gain of chromosome 20 and loss of the extra copy of chromosome 17 or loss of 17p. No mutations of the *p53* gene have been observed in this subtype, suggesting that the *p53* gene most likely does not play an important role in the progression of chromophilic/papillary RCC. Other genetic changes

which have been associated with tumor progression in chromophilic/papillary RCC, aside from those related to the transition from adenomas into carcinomas, are loss of 6q, 9, 11, 14q, and 21 and gain of chromosome 8 [26,76,107,108].

A small subset of chromophilic RCC is characterized by translocations of the X chromosome at Xp11.2. Thusfar, a specific t(X;1)(p11.2;q21) has been observed in eight cases, in some of which as the sole cytogenetic aberration. In others it is accompanied by other changes, including trisomy 17. The genes involved in the t(X;1)(p11.2;q21) have recently been cloned [121,122] and it has been shown that the translocation results in a fusion of the transcription factor TFE3 on the X chromosome, with a novel gene, designated *PRCC*, on chromosome 1. Variants of this translocation have also been described. A literature review of these cases is summarized in chapter 3.2.2. Chromophilic tumors showing Xp11.2 translocations have a tendency to occur in children and young adults. Morphologically these tumors are characterized by the deposition of fat and glycogen in their cytoplasm to an extent that the cells resemble large clear cells. A papillary growth pattern, usually found in chromophilic/papillary tumors, may not predominate in these neoplasms, leading to a misdiagnosis of clear cell RCC, when the commonly accepted criteria of the WHO are applied. A diagnosis based on cell type or genetic constitution would correctly point to a chromophilic variant. In Figure 1 these tumors are depicted as a distinct entity, but, since some of them also show trisomy 17 in addition to an Xp11.2 translocation, they may arise through an "ordinary" chromophilic/papillary adenoma stage, as is marked by an arrow.

Comments on the above mentioned findings:

Although clear cell RCC and chromophilic/papillary RCC both are derived from cells of the same part of the renal tubule, and have a similar antigenic phenotype, the differences in genetic changes associated with the development of these neoplasms suggest that their oncogenesis is different as well as their histogenesis. A major contributing factor to this difference might be that clear cell RCC arises from mature renal tubular cells, whereas chromophilic/papillary tumors most likely have an embryonal origin. The fact that mixed tumors, comprising both clear cell and chromophilic/papillary areas, exist does not contradict this assumption. Genetic analysis of these tumors reveal the typical chromosome changes of the respective tumor types ([50], and unpublished observations). A number of additional changes, mostly related to tumor progression, are similar for both subtypes [26,28,46,53,104-108]. Common changes observed in both subtypes are loss of 6q, 9, 11, 14q, 17p, and gain of chromosomes 12 and 20. Whether shared, progression related, genetic changes, as observed in these subtypes, point to a partly common oncogenetic pathway because of their common origin, i.e. the metanephros, has to be elucidated.

Renal oncocytomas, chromophobe RCC and Duct Bellini carcinomas:

Renal oncocytoma or renal cell adenoma of the oncocytic type, comprising approximately 4% of RCC, is an essentially benign neoplasm, although cases with local or distant metastasis have been described [5]. The male to female ratio is 2.5:1. Renal oncocytomas are solitary, in rare instances multiple, well circumscribed, slightly lobulated solid tumors with a tan-brown cut surface and larger tumors exhibit a stellate central scar. Focal hemorrhage and invasion of adjacent

structures may be present, but these tumors do not exhibit necrosis [24]. Microscopically, the tumor consists of cells with abundant granular eosinophilic cytoplasm. The centrally located nuclei are generally round and vesicular, but focal areas may have marked nuclear atypia, probably as a result of polyploidization. The cells are usually arranged into solid nests (acinar growth pattern) and sheets of trabeculae that are separated by loose edematous fibrous stroma. Ultrastructurally the cytoplasm is packed with numerous round mitochondria, rich in cristae. The mitochondria of oncocytomas are larger than the mitochondria of other renal cell neoplasms.
Renal oncocytomas find their origin in the intercalated cells of the collecting tubule, which is substantiated by the shared expression of carbonic anhydrase C (CAC) and band-3 protein. However, tumor associated loss of antigen might occur [152]. Therefore not all oncocytic cells are positive for the above mentioned antigens.

Little is known about the genetic changes responsible for the development of renal oncocytomas, but different authors have observed alterations of the mitochondrial DNA. Telomere shortening and telomeric associations have been observed in renal oncocytomas [73]. Cytogenetic analysis of these neoplams has revealed a variety of chromosomal patterns, suggesting the existence of distinct subsets [26,67,69-71,132-134,136,138,146]. Several cases display mixed populations of cells with normal and abnormal karyotypes, which fail to show any cytogenetic similarity [26]. Others seem to reveal a consistent pattern of genetic changes. They can be divided into 1) tumors showing the combined loss of chromosomes 1 and X/Y and 2) tumors revealing translocations involving chromosome 11, with breakpoint 11q13. Recently Sinke et al [147] mapped this oncocytoma specific 11q13 breakpoint between D11S443/D11S146 and the BCL1 locus, using two t(5;11)-positive renal oncocytomas. The 11q13 breakpoint of a third case, revealing a t(9;11)(p23;q13) was found to be located in the same region (unpublished data). Tumor-specific translocations have been described in solid tumors, some of which result in the production of chimeric transcription factors. A notable example is the finding of a specific (X;1) translocation in a subset of chromophilic RCC (described above). A similar mechanism might play a role in the development of the oncocytomas characterized by translocations involving breakpoint 11q13. The fusion of 11q13 with either 5q35 or 9p23 may result in a new fusion gene, the expression of which is responsible for uncontrolled growth. Direct activation of a putative oncogene by juxtaposition to active promotor/enhancer sequences is another possible mechanism.
Loss of chromosomes 1 and X or Y probably results in loss of tumor suppressor gene(s) responsible for the development of this subset of oncocytomas [167]. Recent findings indiciate that especially loss of 1p is important in these neoplasms, suggesting the presence of a tumor suppressor gene at this chromosomal region [149]. No morphological differences have been observed between the genetically different subsets of oncocytomas. Nevertheless, the observed distinct genetic changes might have a biological meaning, one of which could be the reported malignant potential of some of the oncocytomas published thusfar. As is discussed in chapter 4.3, a subset of renal oncocytomas might represent the benign counterpart of chromophobe carcinomas. A proposed adenoma carcinoma sequence for these neoplasms will be presented in detail in one of the sections below.

Chromophobe RCC was first described in humans by Thoenes et al. [124]. This subtype accounts for 2-5% of cases and no male preponderance is found [130]. As holds for renal

oncocytomas these tumors find their origin in the intercalated cells of the collecting tubules. Depending on their size, chromophobe tumors consist of one or more solid tumor nodules with a slightly lobulated surface. The cut surface appears homogeneously orange. An uniformly pale cut surface interspersed with a few hemorrhages is a very characteristic gross feature of the well differentiated tumor type, whereas a slightly brown-colored cut surface is usually associated with less differentiated tumors. Microscopically, the basic chromophobe cell type is characterized by large polygonal cells with a transparent, not clear, but slightly reticulated cytoplasm. The nuclei are central or slightly eccentric and usually moderate or substantial in size with marked variation in a single tumor. Clearly recognizable nucleoli are present. Ultrastructurally the cytoplasm of chromophobe cells is crowded with glycogen deposits and numerous, sometimes invaginated, vesicles that resemble those of a subset of intercalated cells of the renal collecting tubule from which chromophobe RCC develops. The origin of the microvesicles is not yet known but the outer membrane of mitochondria have been proposed to be a probable source [168]. A few glycogen particles are found free in the cytoplasm, between the microvesicles and a few mitochondria with a reasonably large number of crista-like or vesicular inner membranes occur loosely scattered at the cell periphery. There are two cytomorphological variants of chromophobe RCC: the typical variant, as described above, and the eosinophilic variant. The latter differs from the typical variant in its higher content of mitochondria, which are also somewhat larger on average. These mitochondria take up an appreciable proportion of the cytoplasm and contain abundant crista-like or vesicular internal membranes. The cytoplasmic microvesicles are present in great numbers, either between the mitochondria or in small mitochondria-free areas. Both variants show a strong positive reaction with Hale's acid iron colloid stain, which is probably the most important diagnostic feature for chromophobe RCC. In addition chromophobe tumors are positive for carbonic andydrase C, but they do not express band-3 protein.

Genetic data on chromophobe RCC are limited, but almost all cases described thusfar are characterized by extensive nonrandom chromosome losses [60,61,71,126,127]. Loss of chromosomes 1, 2, 6, 10, 13, 17, 21, and X/Y is consistently found in these cases. Telomeric associations and telomere shortening have also been observed [73]. Furthermore, molecular analysis of this subtype has revealed gross alterations of the mitochondrial DNA (mtDNA) [54]. Genetic changes related to tumor progression and metastatic behavior within chromophobe carcinomas are not yet known. Thusfar only one cytogenetically analyzed case of a chromophobe metastasis has been described (see chapter 4.1). In this case, next to the extensive chromosome losses specifically assigned to the chromophobe subtype, also structural changes were observed. Whether or not these changes are related to metastatic growth has to await further studies in RCC metastases. Therefore they are not included in Figure 1.

Duct Bellini carcinomas, or collecting duct carcinomas, are rare tumors representing approx 1% of all RCC. They find their origin in the principal cells of the collecting duct system. At presentation, these tumors are usually smaller than other RCC subtypes, but they appear to be aggressive neoplasms, often with metastatic disease at diagnosis, and rapid progression despite surgical intervention. Duct Bellini carcinomas are usually localized to the renal medulla with distortion of the pelvicalyceal system, often with irregular extensions into the adjacent renal cortex.

They generally are white colored and firm at cut surface and lack necrosis and hemorrhage. The growth pattern is mainly tubular combined with a microcystic, pseudopapillary, and solid pattern. The basic cell type exhibits medium-sized tumor cells with a basophilic, sometimes light cytoplasm due to pronounced formation of endoplasmic reticulum and glycogen deposits. Anaplastic nuclei are usually found.
Genetic data on Duct Bellini carcinomas is limited, but these tumors frequently show an aneuploid DNA content. Cytogenetic data published on a few cases thusfar have revealed conflicting results [63]. Molecular analysis has shown that 8p and 13q are frequently lost in these neoplasms [64]. In a recent study, loss of chromosome 1, region 1q32.1-q32.2, appeared to be a consistent finding in Duct Bellini carcinomas [169]. Therefore tumor suppressor genes located at these three chromosome regions might be involved in the development of Duct Bellini carcinoma.

A proposed adenoma-carcinoma sequence for chromophobe RCC

Comparing the morphologic and genetic features of chromophobe carcinomas and renal oncocytomas, a marked degree of similarity is observed, which is not surprising since they have the same progenitor. The intercalated cells of the collecting tubule give rise to both tumortypes, reflected by the shared expression of carbonic anhydrase C. The lack of band 3 protein expression in chromophobe RCC might be due to tumor related loss of antigen. Oncocytomas and the eosinophilic variant of chromophobe RCC show an accumulation of enlarged and morphologically altered mitochondria. Alterations of the mitochondrial DNA (mtDNA) have been observed in chromophobe carcinomas as well as in renal oncocytomas. The characteristic microvesicles, said to be exclusive for chromophobe RCC, have been observed in small amounts in some oncocytomas (Störkel, personal communication), but their origin is presently unknown. Telomeric associations and telomere shortening have been observed in both subtypes. Comparison of the genetic profile of oncocytomas with chromophobe carcinomas reveales that loss of chromosomes 1 and X/Y is shared by both. In addition to loss of chromosomes 1 and X/Y, chromophobe carcinomas show extensive nonrandom whole chromosome losses, involving chromosomes 2, 6, 10, 13, 17, and 21.
The above mentioned findings suggest that chromophobe carcinomas and renal oncocytomas are closely related entities. As is depicted in Figure 2, renal oncocytomas characterized by the combined loss of chromosomes 1 and X/Y might be chromophobe adenomas, explaining why occasionally oncocytomas show a malignant behavior. MtDNA changes and loss of chromosomes 1 and X/Y may be the first oncogenetic steps in their development. Progression from chromophobe adenomas via eosinophilic chromophobe carcinomas to typical chromophobe carcinomas most likely occurs through subsequent chromosome losses, involving chromosomes 2, 6, 10, 13, 17, and 21, and additional mtDNA changes. The latter might result in a sequential breakdown of mitochondria. In addition, characteristic microvesicles accumulate in the cytoplasm of the cells.
Evidence substantiating this proposed oncogenetic pathway are 1) the finding of an oncocytoma containing chromophobe cell nests [151], pointing to a possible transition between both tumor types, 2) the cytogenetic analysis of a case of oncocytoma revealing multiple chromosome losses, among which chromosomes 1, 2, 6, 17, 21, and Y [154], and 3) LOH studies, showing loss of chromosome regions 1p, 2pq, 13q, 17pq, Xpq, in one case of oncocytoma, and loss of 1pq, and

21q in another [149], abnormalities specifically assigned to chromophobe carcinomas (see also chapter 4.3).

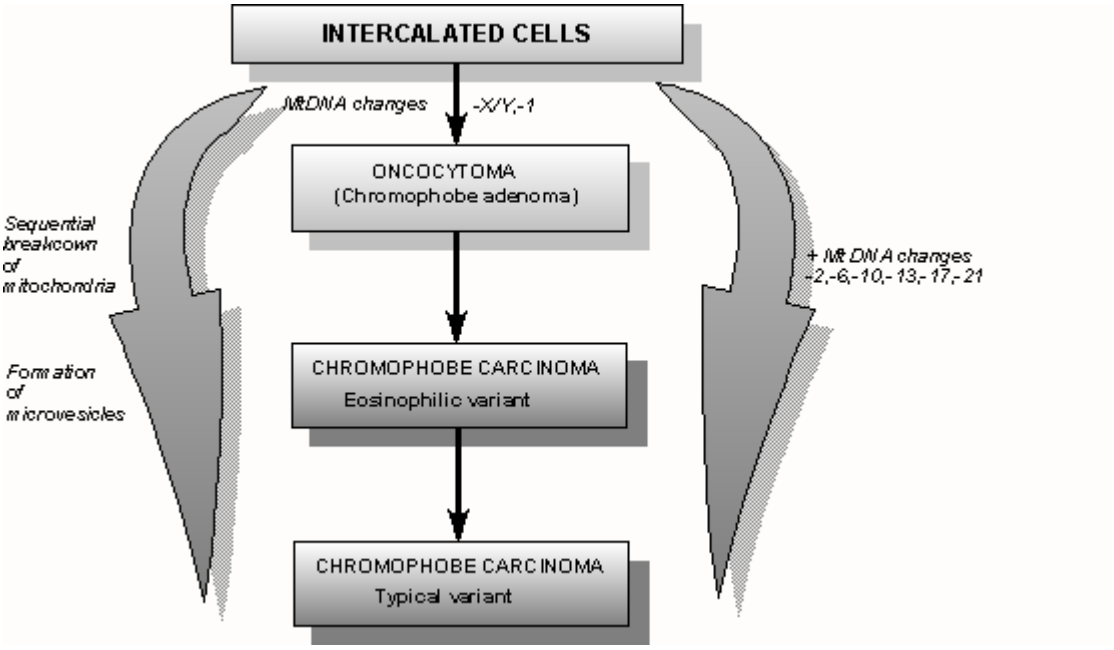


Figure 2: Proposed oncogenetic pathway for chromophobe RCC

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Cytogenetic and molecular genetic studies of RCC have shown that these tumors can be divided into genetically distinct entities. A specific combination of genetic changes marks each subtype, whereas other changes have been related to progression to a more malignant phenotype. There is indeed a close relationship between morphological subtyping according to Thoenes and Störkel, and genetic subtyping of RCC. However, the genetic profile of renal cancers enholds much more information than a morphological subtyping can provide. Renal cell adenomas are difficult, maybe even impossible, to recognize on histological grounds, and the size of a given tumor, often used as a diagnostic criterium, might not be representative for its clinical behavior. The recognition of a distinct adenoma stage in clear cell RCC, characterized by a single deletion in either 3p25 or 3p12-14 (depicted as 3p- in Figure 1) is therefore a tremendous step forward in achieving a proper diagnosis [44]. The same holds for chromophilic adenomas, revealing a -Y,+7,+17 chromosomal pattern without additional changes. The proposed embryonal origin for sporadic chromophilic tumors (chapter 3.1.2), and the observed differences between familial and sporadic cases of this subtype are major contributions to the proposed onco-developmental pathway of these tumors. However, since we do not know, at present, which genetic events, other than -Y, +7, +17, are

involved in the development of familial cases of chromophilic RCC, and whether they also contribute to the development of sporadic cases, familial chromophilic RCC is not mentioned as a separate entity in Figure 1. The proposed adenoma-carcinoma sequence for chromophobe RCC (depicted in detail in Figure 2), reassigning a subset of oncocytomas to the chromophobe subtype, might have diagnostic and prognostic consequences, and also contributes to our understanding of its oncogenesis (see chapter 4.3).

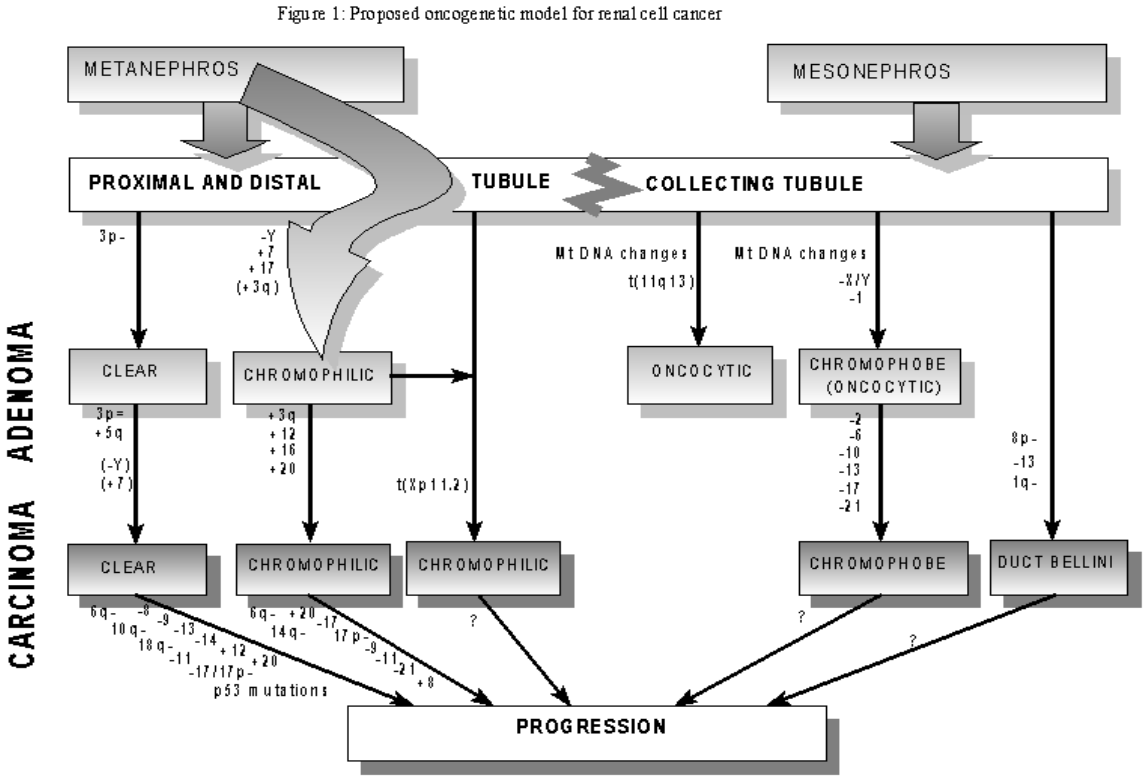
Tumor progression is the result of an accumulation of genetic changes. Genetic changes related to this process, however, do not occur randomly and their appearance may depend on the primary aberration and on the specific tumor type involved. The knowledge of specific progression related genetic changes may provide valuable prognostic information. In RCC several genetic events have been associated with the progression of clear cell and chromophilic tumors, some of which seem to be unique for one of the subtypes, whereas others are shared by both. Common progression related genetic changes are loss of 6q, 9, 11, 14q, 17p, and gain of chromosomes 12 and 20. In addition, clear cell RCC specifically shows gain of 5q, loss of 8p, 10q, 13, and 18q, and mutations of the *p53* gene, whereas the chromophilic/papillary subtype shows gain of 3q, 8, and 16, and loss of 21 (see also chapters 2, 3.1.1 and 3.1.3). No pertinent data are available on progression related genetic changes in other subtypes of RCC thusfar.

Sarcomatoid transformation is an ultimate form of tumorprogression in RCC and may occur in any of the subtypes. The diagnosis of sarcomatoid RCC on morphological grounds may be difficult and incorrect, since the histologic appearance of the carcinoid areas, on which these tumors usually are judged, might not represent the sarcomatoid part of the tumor (see chapter 3.1.3). Assessment of the genetic profile of these tumors may prove to be a valuable diagnostic tool as to tumor subtype, but also in distinguishing sarcomatoid RCC from true renal sarcomas.

Taken together, the genetic constitution of the different subtypes of RCC, as shown in Figure 1, enhold valuable diagnostic, and prognostic information, and genetic analysis of RCC is therefore important for the individual patient affected with this disease. Furthermore, assessment of the distinct genetic profiles observed in RCC has improved our understanding of its oncogenesis and of pathogenetic relationships between tumor subtypes. Presently, the diagnosis of RCC is mainly based on histopathological examination. Considering the close relation between genetic subtyping of RCC and the morphological classification of Thoenes and Störkel, we feel that this classification should be adapted in routine pathologic examination of these neoplasms. However, for the diagnosis of a renal adenoma, either clear, chromophilic/papillary, or oncocytic, genetic analysis is mandatory. In the near future the morphological diagnosis will be increasingly accompanied by cytogenetic and molecular genetic methods, improving the diagnosis, and having prognostic significance. Once the genes, involved in the development and progression of the different subtypes of RCC, have been identified, genetic analysis might be transferred to routine pathology and used in the clinical management of individual patients. Furthermore, identification of the as yet unknown genes, and their encoded proteins, may form the basis for new and improved therapies in the future.

Summary of Figure 1:

A PROPOSED ONCOGENETIC MODEL FOR RENAL CELL CANCER



The compiled morphologic and genetic data of RCC extracted from our survey and from the literature is depicted in a proposed oncogenetic model for RCC (Figure 1). The white bar represents the normal mature renal tubular system with on the left the proximal and distal tubule, and on the right the collecting tubule. Both are separated from eachother by a grey line, indicating their different embryonal origin, i.e. the metanephros and the mesonephros respectively. In the horizontal lane below that, the adenoma stages of the different subtypes of RCC are given. Progression to a carcinoma stage and subsequent progression to a higher grade are indicated in vertical direction. Clear cell and chromophilic/papillary tumors arise from the proximal/distal tubule, whereas renal oncocytomas, chromophobe carcinomas, and Duct Bellini carcinomas find their origin in the collecting tubule. The genetic changes, known thusfar to be involved in the different tumor subtypes and progression stages, are given. Clear cell adenomas are characterized by a single 3p deletion, either in 3p25 or 3p12-14, depicted as 3p- in Figure 1. Progression to clear cell carcinomas is associated with a subsequent deletion of 3p21 (3p= in Figure 1). Trisomy 5q is

another frequent finding in clear cell carcinomas, and occurs independent of tumor grade. Trisomy 7 and loss of the Y chromosome are placed between brackets, since these genetic changes have been found in similar frequencies in normal kidney tissue, and their contribution to the oncogenesis of clear cell carcinomas is therefore debatable. Genetic changes reflecting a higher grade in clear cell carcinomas are: loss of 6q, 8, 9, 10q, 11, 13, 14, 17(p), and 18q, gain of chromosomes 12 and 20 and mutations of the *p53* gene. Chromophilic/papillary adenomas are characterized by trisomy 7 and 17 and loss of the Y chromosome. In some adenomas also trisomy of 3q is observed, but this might be an early sign of malignant transformation, indicated as (+3q) in Figure 1. The big arrow omitting the mature renal tubular stage, reflects the proposed embryonal origin of these neoplasms (chapter 3.1.2). Chromophilic/papillary carcinomas reveal additional trisomies of chromosomes 3q, 12, 16, and 20, and progression to a higher grade is associated with los of 6q, 9, 11, 14q, 17(p), and 21, and gain of chromosome 8. A small subset of chromophilic tumors is characterized by translocations involving chromosome X, with breakpoint Xp11.2. In some of these tumors also trisomy of 7 and 17 is observed. "True" renal oncocytomas are characterized by translocations involving chromosome 11, breakpoint 11q13, and changes of the mitochondrial DNA (mtDNA). Chromophobe adenomas reveal the combined loss of chromosomes 1 and X/Y, and mtDNA changes. Chromophobe carcinomas show additional loss of chromosomes 2, 6, 10, 13, 17, and 21. Duct Bellini carcinomas are characterized by loss of 1q, 8p, and 13. Genetic changes associated with a higher grade in chromophobe carcinomas and Duct Bellini carcinomas have not been assessed.

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NEDERLANDSE SAMENVATTING VOOR DE NIET INGEWIJDE LEZER

Chromosomen, genen, en hun relatie met kanker

Leven en dood zijn onlosmakelijk met elkaar verbonden. Dit geldt voor ons als individuen, maar ook voor de circa honderd biljoen cellen waaruit ons lichaam bestaat. In deze complexe celpopulatie is de verhouding tussen celgroei en celsterfte strikt gereguleerd. Van conceptie tot volwassenheid is een gereguleerde toename van het aantal cellen noodzakelijk. Naast de toename van het aantal cellen vindt specialisatie (differentiatie) plaats. Op deze manier worden verschillende weefsels met elk hun specifieke functies gevormd In het volwassen individu moeten celsterfte en celdeling/differentiatie in evenwicht zijn. Celdeling is hier nodig om afgestorven cellen te vervangen, bijvoorbeeld bij verwondingen of infecties, maar ook om constant regenererende weefsels zoals darmen, huid en bloedcellen op peil te houden.

Alle informatie die nodig is om de processen van celgroei en differentiatie uit te voeren ligt opgeslagen het erfelijke materiaal (DNA) in de celkern. Dit DNA is gelegen in de chromosomen, en de gebieden in het DNA die coderen voor een bepaalde functie worden de genen genoemd. Slechts een klein gedeelte van ons totale DNA is betrokken bij deze coderende functie.

Een humane cel heeft 46 chromosomen, 44 autosomen en 2 geslachtschromosomen (de geslachtschromosomen bij vrouwen bestaan uit twee X chromosomen, terwijl mannen een X en een Y chromosoom hebben). De autosomen zijn in tweevoud aanwezig en zijn genummerd van chromosoom 1 tot en met chromosoom 22. Tijdens de celdeling wordt het DNA verdubbeld (DNA replicatie) en aan elk van de beide dochtercellen wordt een replica doorgegeven. Elke cel van ons lichaam bevat dus dezelfde genetische informatie. Specialisatie processen, noodzakelijk om elke cel zijn eigen specifieke functie te laten uitvoeren zorgen ervoor dat slechts die genen die in een specifiek weefseltype een functie hebben tot expressie komen.

De genen die betrokken zijn bij de normale celding kunnen onderverdeeld worden in drie groepen. Proto-oncogenen zijn genen die een cel aanzetten tot delen. Tumor suppressor genen zijn genen die een remmende werking op de celdeling en celgroei hebben. Stabiliteits genen waarborgen de integriteit van het genoom en zijn onder andere betrokken bij DNA herstel en correcte uitvoering van de DNA replicatie.

Veranderingen in de bovengenoemde genen kunnen resulteren in een ongeremde groei van cellen, en dus kanker veroorzaken. Deze veranderingen kunnen ontstaan door invloeden van buitenaf (bijvoorbeeld door straling of door contact met chemische verbindingen), maar ook door fouten, ontstaan tijdens de celdeling, die niet (goed) hersteld worden.

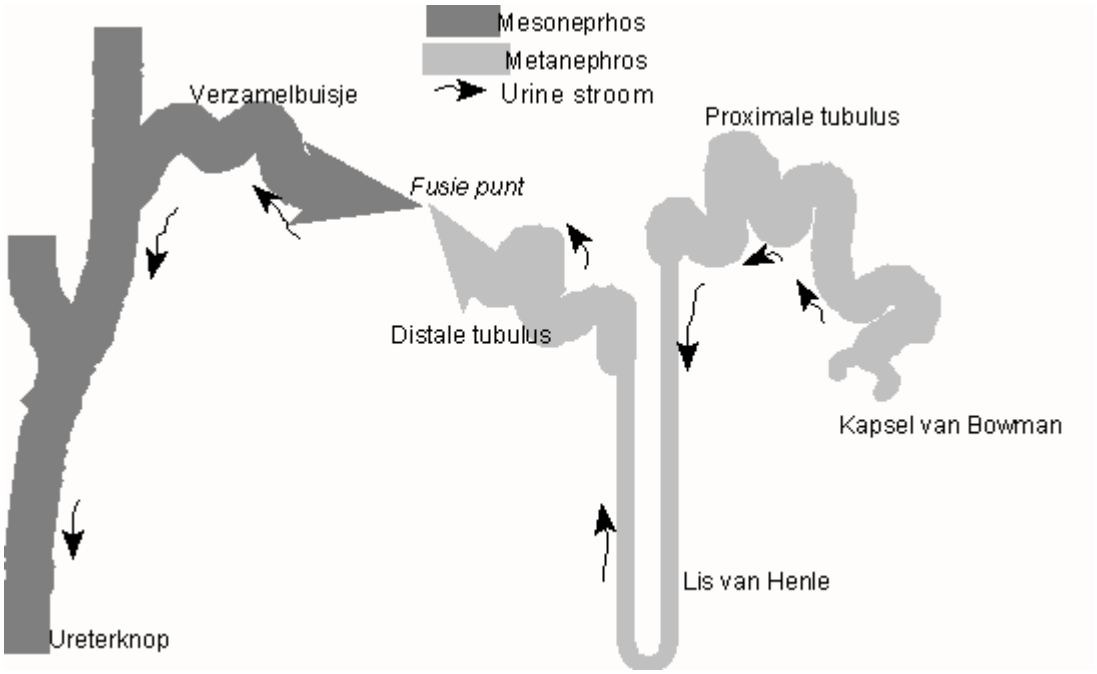
Kanker ontstaat in principe uit één cel en is dus monoklonaal. Aangezien een cel beschikt over vele mogelijkheden om de celdeling te reguleren, zijn meerdere mutaties nodig om van een normale cel een kankercel te maken. Het ontstaan van kanker is dus een meerstaps proces, veroorzaakt door een opeenstapeling van genetische veranderingen, die cel op cel worden doorgegeven tijdens de celdeling. Naarmate het aantal mutaties toeneemt, kan de cel meer kwaadaardige eigenschappen krijgen (progressie) die zorgen voor een nog snellere groei en het uitzaaiien van de tumor naar andere weefsels (metastasering).

Door deze veranderingen in het DNA te onderzoeken kunnen we iets te weten komen over de mechanismen die ten grondslag liggen aan het onstaan en de progressie van kanker. Dit kan door de chromosomen te bestuderen (cytogenetisch onderzoek). Chromosomen, die zichtbaar gemaakt kunnen worden tijdens bepaalde stadia van de celdeling, hebben een lange en een korte

arm (q en p arm) gescheiden door een centromeer. Ten gevolge van speciale kleuringstechnieken vertoont elk chromosoom een uniek bandenpatroon waaraan de individuele chromosomen kunnen worden herkend. De verschillende chromosoombanden zijn genummerd volgens internationaal geldende regels. Op grond van bovenstaande kenmerken kunnen afwijkingen van het normale patroon herkend en benoemd worden. Genetische veranderingen kunnen ook op gen nivo bestudeerd worden door rechtstreeks naar veranderingen in het DNA te kijken (moleculair genetisch onderzoek). Beide technieken hebben vóór en nadelen en een combinatie van de twee levert het meest complete beeld van de genetische veranderingen die ten grondslag liggen aan het bestudeerde type kanker.

Structuur en functie van de nieren

De nieren zijn twee boonvormige organen die gemiddeld 12 cm in lengte, 6 cm in breedte en 2,5 cm in doorsnede zijn. Ruim 1700 liter bloed passeert dagelijks de nieren, en de afvalstoffen hieruit worden omgezet in ongeveer 1 liter geconcentreerde urine. Op deze manier zorgen de nieren ervoor dat deze afval stoffen van ons lichaam worden uitgescheiden. Ze regelen de vochthuishouding van ons lichaam en de zuurtegraad van het plasma. Bovendien scheiden ze hormonen uit, zoals erythropoetine, renine en prostaglandine.



Figuur 1: Schematische weergave van de opbouw van een uitscheidingsorgaan

De nier is opgebouwd uit een groot aantal uitscheidingsorganen, die ieder bestaan uit een nefron en een verzamelbuisje. Beide structuren ontwikkelen zich apart tijdens de embryogenese, waarna ze met elkaar fuseren en zo één uitscheidingsorgaan vormen (zie Figuur 1).
De verzamelbuisjes ontstaan uit de ureterknop (mesonephros) en de nefronen worden gevormd uit het metanefrogene mesoderm (metanephros). Een nefron bestaat uit het kapsel van Bowman (die de glomerulus omsluit), de proximale tubulus, de lis van Henle, en de distale tubulus. De laatste fuseert met het proximale uiteinde van een verzamelbuisje.
De urine stroom loopt vanaf het kapsel van Bowman (glomerulus) via de proximale tubulus, lis van Henle, distale tubulus, en het verzamelbuisje naar de ureter, om vervolgens in de blaas te eindigen. Langs dit traject worden onder andere kostbare eiwitten en aminozuren geresorbeerd en de juiste zout en zuurgraad van de urine geregeld. Verschillende celtypen, die elk hun eigen plaats in elk van de uitscheidingsorganen hebben, zorgen voor het uitvoeren van al deze functies.

Nierkanker

Nierkanker is een heterogene groep van kankersoorten die voorkomt met een frequentie van 1 tot 2 op de 100.000 personen per jaar. Ongeveer 2% van alle gediagnostiseerde tumoren betreft nierkanker. Dit proefschrift handelt over de zogenaamde adenocarcinomen van de nier die ongeveer 85% van alle gevallen van nierkanker beslaan. Nierkanker is voornamelijk een ziekte van ouderen en wordt meestal na het 50^{e} levensjaar gediagnostiseerd. Deze ziekte komt twee keer zo vaak voor bij mannen als bij vrouwen door vooralsnog onbekende oorzaken.

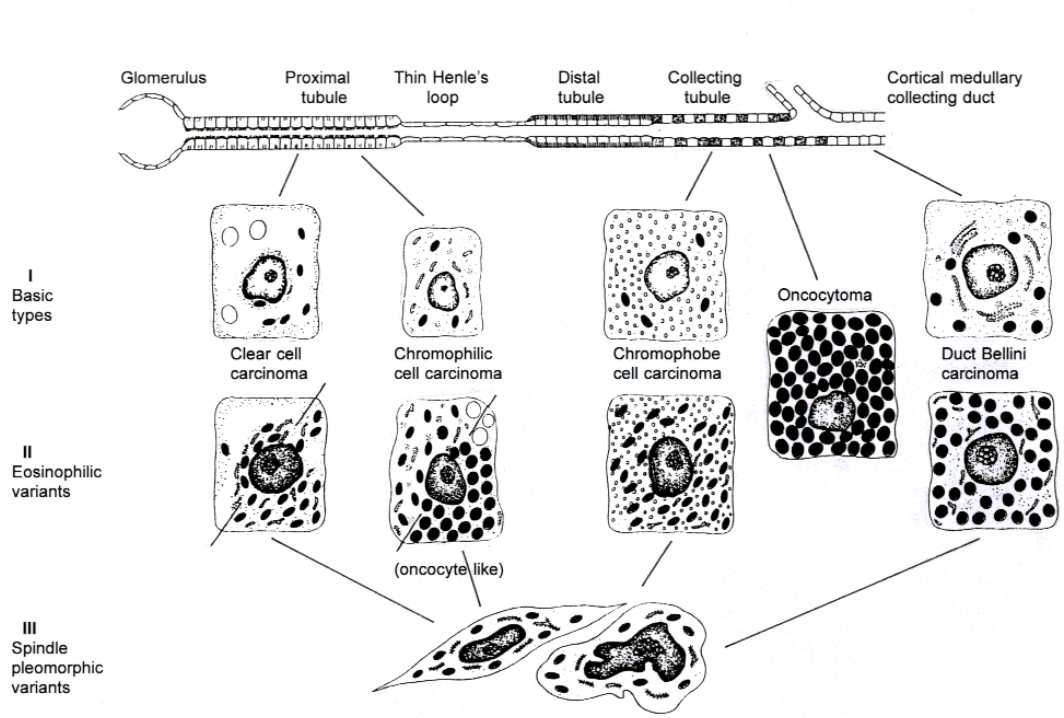
De meeste gevallen van nierkanker zijn sporadisch in oorsprong, slechts 2% van alle gevallen van nierkanker is erfelijk. Erfelijke nierkanker komt voor bij mensen met het Von Hippel Lindau (VHL) syndroom. Patienten met het VHL syndroom hebben een verhoogde kans op het krijgen van allerlei tumoren waaronder nierkanker. Ook zijn er families waarin een constitutionele translocatie (een uitwisseling van stukken van chromosomen die bij deze patienten in alle DNA bevattende lichaamscellen voorkomt en overgeërfd kan worden van ouder op kind) voorkomt waarbij de korte arm van chromosoom 3 (3p) betrokken is. Familieleden die drager zijn van deze translocatie krijgen bijna altijd nierkanker. Niet erfelijke risicofactoren die bijdragen tot het ontstaan van nierkanker zijn roken, voornamelijk bij mannen, en extreem overgewicht (obesitas), voornamelijk bij vrouwen. Ook is een relatie aangetoond tussen het ontstaan van nierkanker en blootstelling aan bepaalde mutagene stoffen, zoals bijvoorbeeld asbest.

Karakteristiek voor nierkanker is het afwezig zijn van specifieke symptomen (zoals bloed in de urine en pijn in de zij) in een vroeg stadium van deze ziekte. Kleine lokale tumoren veroorzaken zelden klachten. Daarom worden veel van deze kankers pas (te) laat ontdekt en heeft 25-30% van de patienten al metastasen (uitzaaiingen) op het moment van diagnose.

De behandeling van nierkanker bestaat vooralsnog uit het verwijderen van de aangedane nier met tumor en omliggende weefsels (radicale nefrectomie). Wanneer de ziekte gemetastaseerd is, zijn er nog weinig mogelijkheden tot behandeling, aangezien chemotherapie, radiotherapie (straling) en immunotherapie weinig of geen effect hebben.

De histologische versus genetische classificatie van nierkanker:

Nierkanker is een heterogene ziekte, wat ook tot uitdrukking komt in hun biologisch gedrag. Dit varieert van goedaardig tot zeer kwaadaardig. Het spreekt voor zich dat voor de individule patient en zijn prognose een juiste diagnose belangrijk is. Niertumoren worden tot nu toe op grond van hun histologie (cel en weefselkenmerken) gediagnostiseerd. De classificatie van de wereld gezondheids organisatie (WHO) wordt het meest gebruikt. Deze verdeelt nierkanker in adenomen (goedaardige tumoren), carcinomen (kwaadaardige tumoren) en overigen, waarbij in de carcinomen nog de tweedeling papillair versus niet papillair, afhankelijk van de groeiwijze van de tumorcellen, genoemd wordt. Deze classificatie is erg summier en doet geen recht aan de grote verscheidenheid van biologische verschijningsvormen van nierkanker die voorkomen. In 1986 is een nieuwe, morfologische classificatie voor nierkanker voorgesteld door Thoenes en Störkel die een meer uitgebreide subtypering toelaat. In deze classificatie worden vijf verschillende typen nierkanker herkend, uitgaande van de verschillende celtypen die aangetroffen worden in de uitscheidingsorganen (zoals hiervoor beschreven). Niertumoren van het heldercellige (clear cell) en het chromofiele type gaan uit van de cellen van het proximale/distale gedeelte van het nefron, oncocytomen en chromophobe niertumoren ontstaan uit de intercalerende cellen van de verzamelbuisjes en niertumoren van het Ductus Bellini type ontstaan uit de zogenaamde "principal cells" van de verzamelbuisjes. Drie verschillende groeipatronen worden herkend: compact, acinair en tubulo papillair. Een schematische weergave van deze classificatie is te zien in Figuur 2.



Figuur 2: Morfologische classificatie van nier cel tumoren volgens Thoenes en Störkel [20].

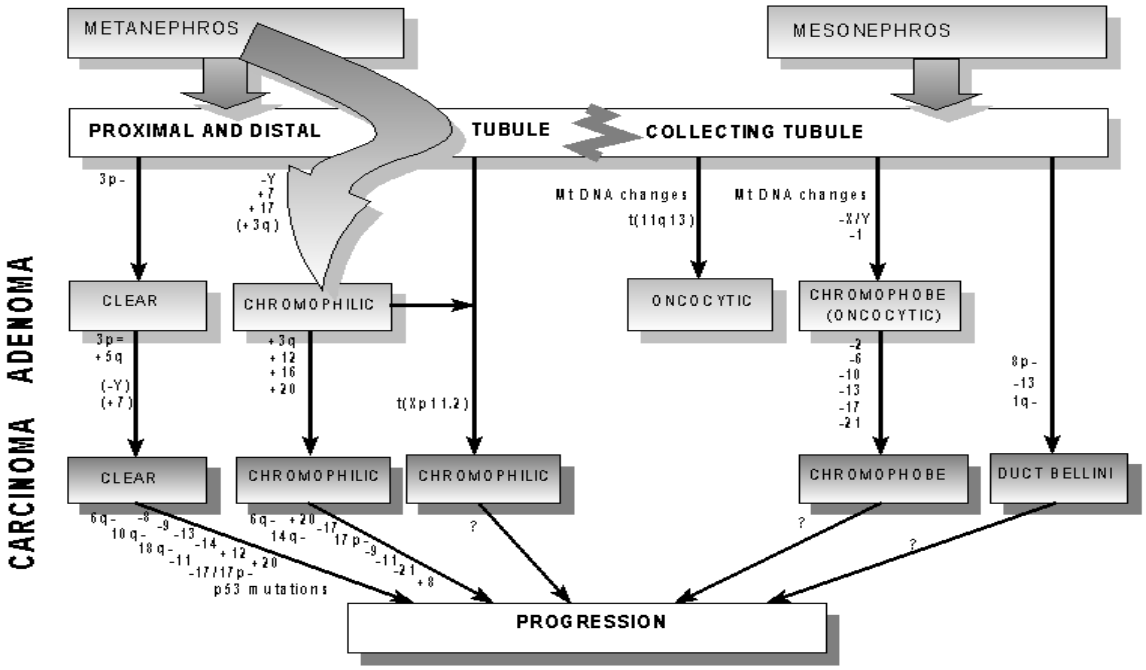
Een belangrijk probleem bij alle op histologie berustende classificatie systemen is dat het uiterlijk en de eigenschappen van de cellen waarop deze classificaties zijn gebaseerd drastisch

kunnen veranderen tijdens tumor progressie. In het algemeen geldt: hoe kwaadaardiger de tumor, hoe minder gelijkenis er is met het oorspronkelijke weefsel. Het stellen van een juiste diagnose op histologische kenmerken is daarom niet altijd gemakkelijk. Aangezien kanker een genetische ziekte van cellen en weefsels is die ontstaat door veranderingen in de chromosomen (genen), zou het mogelijk kunnen zijn op grond van de genetische veranderingen deze tumoren te classificeren. Het grote voordeel van een genetische classificatie is dat genetische veranderingen, in tegenstelling tot histologische kenmerken, constant zijn tijdens tumor progressie. Bovendien is het mogelijk dat een genetische classificatie een meer gedetailleerde subtypering oplevert, waarbij ook verbanden tussen de subtypen onderling en verschillen binnen één subtype kunnen worden aangetoond. Genetisch onderzoek van nierkanker is niet alleen belangrijk voor een juiste diagnose. Kennis omtrent de genetische mechanismen die ten grondslag liggen aan het ontstaan en de progressie van de verschillende subtypen nierkanker kan belangrijk zijn voor het ontwikkelen van nieuwe therapieën in de toekomst.

Uitgaande van de histologische subtypen zoals in de classificatie van Thoenes en Störkel zijn beschreven, hebben wij de genetische veranderingen in deze tumoren bestudeerd. Er is aangetoond dat elk van de verschillende typen nierkanker, zoals beschreven in deze classificatie, karakteristieke genetische veranderingen laat zien. Ook zijn er relaties aangetoond tussen specifieke afwijkingen en progressie van bepaalde subtypen. Door onze gegevens en de gegevens uit de literatuur over deze tumoren te combineren hebben we een oncogenetisch model voor nierkanker ontwikkeld, weergegeven in Figuur 3. Het volwassen uitscheidingsorgaan is weergegeven als een witte balk waarin de proximale en distale tubulus, en het verzamelbuisje herkend worden. Daarboven worden de embryonale weefsels, waaruit deze structuren ontstaan, genoemd. Onder het uitscheidingsorgaan zijn de adenoom stadia van de verschillende subtypen niertumoren weergegeven, gerelateerd aan hun voorlopercel. In verticale richting zien we progressie naar het carcinoomstadium en vervolgens progressie naar een hogere maligniteitsgraad. De genetische veranderingen zijn in dit model per subtype en maligniteitsgraad weergegeven en voor zover bekend zijn ook belangrijke chromosoom gebieden en chromosoom breukpunten genoemd. Niertumoren van het clear cell type worden gekarakteriseerd door verlies van stukjes van de korte arm van chromosoom 3 (deleties). Clear cell adenomen hebben slechts één deletie, aangeduid met 3p- in Figuur 3, terwijl clear cell carcinomen minimaal twee deleties laten zien (3p= in Figuur 3). Progressie van dit subtype niertumoren gaat gepaard met het verkrijgen van additionele afwijkingen, zoals verlies van de chromosomen (of delen daarvan) 8, 9, 13, 14, 6q, 10q, 11, 17(p), 18q, winst van chromosomen 12 en 20, en mutaties in het *p53* gen, een gen dat een rol speelt bij het herstellen van DNA schade.

In chromofiele tumoren is een specifieke combinatie van chromosomen in drievoud aanwezig. Het adenoom stadium van dit type laat winst van chromosomen 7 en 17 zien, samen met verlies van het Y chromosoom bij mannen, terwijl progressie naar een carcinoom stadium gepaard gaat met winst van de chromosomen 3q, 12, 16, en/of 20. Verdere progressie (Figuur 3, onderaan) gaat gepaard met verlies van (de korte arm van) chromosoom 17 (17p), terwijl winst van chromosoom 20 ook hier een rol kan spelen

Figuur 3: Oncogenetisch model voor nierkanker



(beschreven in hoofdstuk 3.1.1). Andere afwijkingen geassocieerd met progressie in dit subtype zijn verlies van de chromosomen 6q, 9, 11, 14, 21, en winst van chromosoom 8. Recent hebben wij aangetoond dat deze tumoren mogelijk een embryonale oorsprong hebben (beschreven in hoofdstuk 3.1.2), dat wil zeggen dat de eerste oncogenetische stap al vóór de geboorte, tijdens de ontwikkeling van de nier, moet zijn ontstaan. In Figuur 3 is dit aangegeven met de grote pijl vanuit het embryonale weefsel (metanephros) naar het chromofiele adenoomstadium.

Een klein aantal chromofiele tumoren heeft een karakteristieke translocatie (verplaatsing van een stuk DNA van het ene naar het andere chromosoom) tussen chromosoom X (breukpunt Xp11.2) en chromosoom 1 (hoofdstuk 3.2). In sommige gevallen is deze translocatie de enige (zichtbare) genetische verandering, in andere gevallen worden ook andere afwijkingen gevonden, o.a winst van chromosomen 7 en 17. Deze tumoren worden gezien als een aparte subgroep van chromofiele tumoren, zoals te zien is in Figuur 3.

Oncocytomen zijn in principe goedaardige tumoren, hoewel in de literatuur metastaserende oncocytomen zijn beschreven. Deze tumoren zijn genetisch heterogeen. Tot nu toe lijken er tenminste twee genetisch verschillende subtypen te bestaan. Eén van deze wordt gekarakteriseerd door translocaties waarbij met name chromosoom 11 (breukpunt 11q13) betrokken is. Het andere subtype laat een combinatie van verlies van chromosomen 1 en X of Y (afhankelijk van het geslacht) zien. Verder hebben deze tumoren veranderingen in het mitochondriale DNA. Chromophobe tumoren worden gekenmerkt door een aanzienlijk verlies van chromosomen, meer specifiek verlies van chromosomen 1, 2, 6, 10, 13, 17, 21, en X of Y, en door veranderingen in het mitochondriale DNA.

Oncocytomen en chromophobe carcinomen ontstaan uit dezelfde voorlopercellen, en ze delen dan ook een aantal eigenschappen. Het grote verschil tussen beide tumoren is dat

oncocytomen in principe goedaardige tumoren zijn en chromophobe carcinomen maligne gedrag vertonen (hoofdstuk 4.1). Op grond van de gelijkenissen tussen beide tumoren (zowel morfologisch als genetisch) en de bevinding dat sommige oncocytomen toch maligne gedrag vertonen, denken wij dat mogelijk die oncocytomen die gekarakteriseerd worden door verlies van de chromosomen 1 en X of Y, geen oncocytomen zijn maar adenomen van het chromophobe subtype (zie hoofdstukken 4.3 en 5). Progressie naar chromophobe carcinomen gaat gepaard met additioneel verlies van de chromosomen 2, 6, 10, 13, 17, en 21.

Weinig is bekend over de genetische veranderingen in nierkanker van het Ductus Bellini type. Uit de literatuur is bekend dat verlies van de korte arm van chromosoom 8, verlies van de lange arm van chromosoom 13, en verlies van de lange arm van chromosoom 1 mogelijk een rol spelen in de oncogenese van dit subtype niertumoren.

Uit bovenstaande gegevens blijkt een duidelijke relatie tussen de histologische subtypering van Thoenes en Störkel en de genetische karakterisering. Daarenboven heeft het bestuderen van de genetische afwijkingen van deze tumoren de classificatie verfijnd. Belangrijke toevoegingen aan de histologische classificatie zijn de bevindingen dat clear cell, chromofiele en chromophobe tumoren een genetisch gedefinieerd adenoom stadium hebben en dat de aanleg om chromofiele tumoren te krijgen mogelijk al vóór de geboorte ontstaat. De genetica kan een belangrijke bijdrage leveren aan de diagnose en prognose, maar verder onderzoek is noodzakelijk teneinde de genen verantwoordelijk voor het ontstaan en de progressie van de verschillende subtypen te identificeren. Deze kennis vormt de basis voor de ontwikkeling van nieuwe therapieën in de toekomst.

CURRICULUM VITAE

28 november 1964:	geboren te Veendam
1 juli 1982:	HAVO diploma. Rijksscholengemeenschap "Winkler Prins" te Veendam
7 juli 1987:	diploma Hoger Laboratoriumonderwijs, Klinische Chemie Rijkshogeschool Groningen te Groningen
1 juli 1987:	Aanstelling als research analist op KWF project: GUKC 87-13 The role of sex of the grafted embryo in determining malignant development of embryo-derived teratomas
1 juli 1989:	Aanstelling als research analist op KWF project: GUKC 88-10 "Relationship between seminoma and non seminoma. Genomic analysis of tumorprogression in testicular germ cell tumors".
1 januari 1992:	Aanstelling als klinisch research analist ten behoeve van patiëntgebonden onderzoek en diagnostiek.

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